Theoretical and experimental approaches for studying factors defining the *Helicobacter pylori*–host relationship

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Mathematical modeling has helped develop hypotheses about the role of microbial and host parameters in the initial and subsequent phases of *Helicobacter pylori* colonization. Transgenic mice have been used to test the hypothesis that the outcome of colonization is influenced by whether bacteria can adhere to available epithelial cell receptors. Complementary use of modeling and experimental approaches should facilitate studies of *H. pylori* pathogenesis.

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Humans must adapt to life in a microbial world. It has been estimated that the total number of microorganisms that colonize our mucosal surfaces exceeds our total number of somatic and germ cells by at least one order of magnitude[1]. The relationships we have with components of our resident microbial societies can, depending upon circumstances, span a continuum from mutually beneficial (symbiotic), to benefiting one partner without necessarily being detrimental to the other (commensal), to benefiting one partner while producing significant loss of fitness in the other (parasitic). In this article, we will consider mutualism to encompass both commensal and symbiotic relationships. *Helicobacter pylori* provides an opportunity to study the foundations of mutualism in the gut, and the conditions that promote a transition from mutualism to parasitism (disease). Relationships involving *H. pylori* are amphibiotic: the potential for harm depends upon the context.

Presently, ~50% of humans harbor *H. pylori* in their stomachs. In most people the time and mode of acquisition of this organism are unknown, although studies suggest that the majority of individuals acquire *H. pylori* in childhood. One current view is that a single predominating strain of *H. pylori* is acquired from another family member[2,3]. Once acquired, *H. pylori* can persist in its gastric habitat for decades. During this time it can undergo considerable genetic evolution, consistent with quasispecies formation[4]. Genetic alterations can arise from point mutations, loss of components of its 31-gene *cag* pathogenicity island (PAI), transposon movement, or acquisition of DNA from other strains that are short- or long-term residents in the stomach[5–7]. These genetic changes could facilitate adaptation to the gastric ecosystem of a particular host, including changes in environmental features brought about by *H. pylori* colonization.

Some of the proposed advantages bestowed by *H. pylori* include resistance to colonization by other pathogens via simple occupancy of the gastric niche, elaboration of antimicrobial peptides, augmentation of acid secretion and/or enhanced immune responsiveness[8,9]. Gastroesophageal reflux disease (GERD) is a prominent risk factor for esophageal metaplasia and adenocarcinoma[10], and it has been suggested that eliminating *H. pylori* might increase the incidence of GERD (Ref. 11).

However, *H. pylori* also produces significant pathology in a subset of its hosts. The cumulative risk of developing peptic ulcers over a 20-year period is three or four times higher in *H. pylori*-positive individuals[12] and epidemiological studies have indicated that *H. pylori* increases the risk of gastric cancer[13]. *H. pylori* appears to emerge as a pathogen from a concurrence of multiple host and microbial factors. Unfortunately, the genetic diversity between isolates of *H. pylori*[6,7] and between humans has impeded efforts to identify the critical determinants of the relationship between *H. pylori* and its host. The purpose of this review is to outline two approaches for

0966-842X/00/$ - see front matter PII: S0966-842X(00)01780-7

addressing this issue. The first involves mathematical modeling of the impact of variations in host and microbial parameters on the initial and steady-state phases of colonization. The second involves genetic manipulation of potential contributing factors in transgenic mice and assessment of the effects on the outcome of colonization. These two approaches are complementary and should be applicable to other host–microbial relationships.

Mathematical modeling studies

Mathematical models of bacterial colonization have helped identify elements of host–microbial interactions that are otherwise experimentally intractable[14]. This is certainly true for *H. pylori*. A review of modeling techniques[14] and full descriptions of models of *H. pylori* colonization can be found elsewhere[15,16]. Here, we focus on two areas in which modeling has helped define the pathophysiology of *H. pylori* colonization: (1) the interplay between different populations of *H. pylori* within the gastric ecosystem and (2) the host response.

Mucus vs adherent epithelial populations

H. pylori can adhere to the gastric epithelium[17–19]. Recent estimates of the population of *H. pylori* in the mucus gel overlying the epithelium range from 10^4 to 10^5 organisms per cubic mm[20,21]. Histological assays indicate that the population adherent to the epithelium ranges from 10^1 to 10^3 organisms per cubic mm[20]. Thus, the adherent population appears to comprise <1% of the total mucus population, suggesting that there could be a limited number of *H. pylori* binding sites or receptors available. Adherent phenotypes could be advantageous for a number of reasons (e.g. proximity to nutrients, a slower rate of washout from the ecosystem or provision of a niche with a more favorable pH), resulting in strong and ongoing selection for optimal adherence phenotypes.

When considering a model of *H. pylori* colonization and persistence, it is necessary to include populations of bacteria that are both free-living in the mucus gel and adherent to epithelial cells (Fig. 1). The model predicts that free-living and adherent *H. pylori* populations achieve persistence, representing 99% and 1% of the total mucus population, respectively. However, the model is able to permit substantial deviation from these values and still maintain steady state. The model incorporates migration of a proportion of the *H. pylori* cells from the mucus to adherent sites on the epithelium. As adherent *H. pylori* divide and the capacity of the sites/receptors is likely to be near saturation, most of the daughter cells will migrate back into the mucus layer. Even though continual exfoliation suggests that there are always available sites, this number will still be limited (i.e. less than infinity) at the site of colonization.

In the model, nutrients are viewed as both necessary and growth limiting. Adherent bacteria are assumed to have a growth advantage based on proximity to epithelial cells (e.g. a higher pH is known to affect *H. pylori* survival[22]). Gastric epithelial cells are sloughed continuously, although at a rate that is slower than the rate of mucus shedding[23]. Microbial location – free living in the mucus or adherent – then determines the corresponding washout (loss) rates.

We have incorporated each of these interactions into a theoretical model describing the dynamic relationship between host and microorganism (Fig. 1a). Once the theoretical model was constructed, it could be translated into a mathematical model. For example, the equation for the rate of change of the adherent bacterial population [A(t)] is:

$$dA(t)/dt = g_A N(t) A(t) - \mu_A A(t) + a M(t)[K - A(t)] - \delta A(t)$$

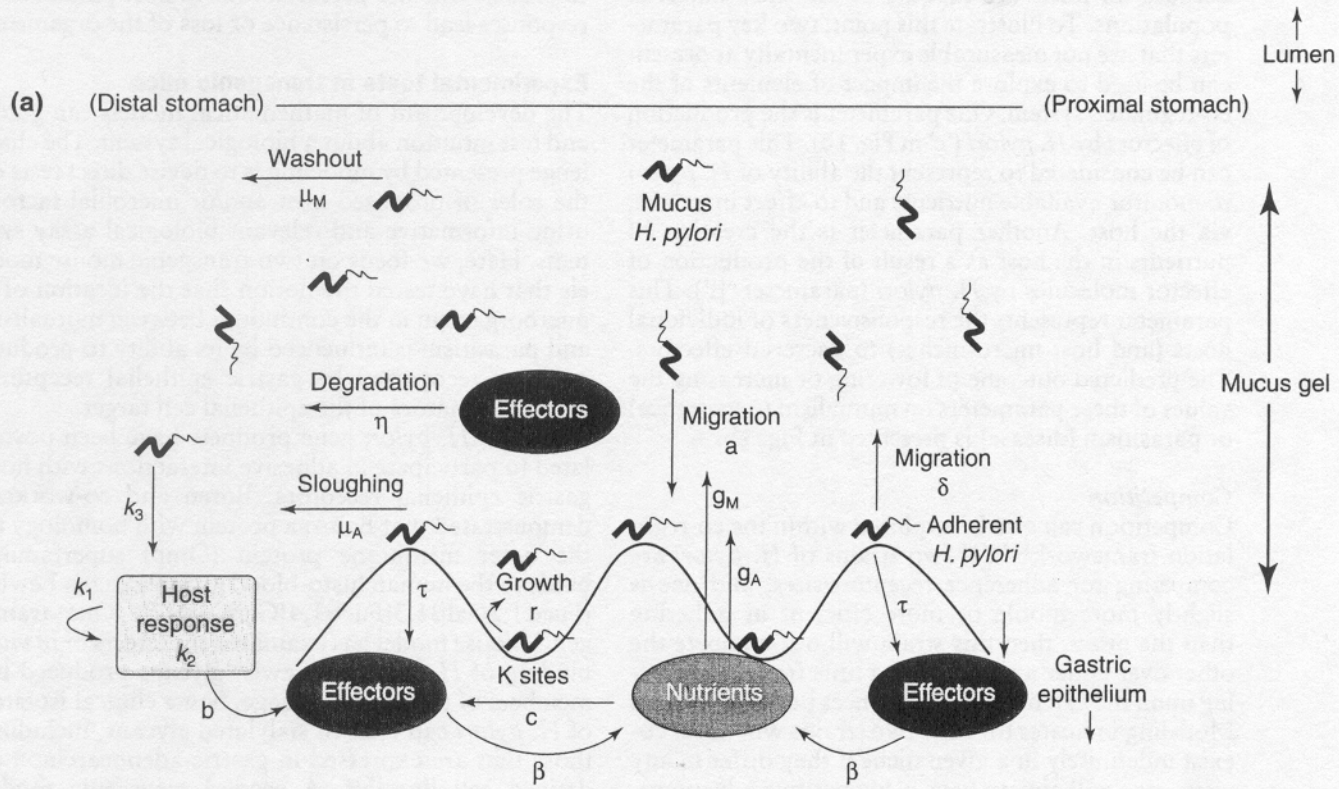
where the first term represents growth at rate g_A as a function of nutrients, N(t), and the second term $[\mu_A A(t)]$ is the loss of *H. pylori* owing to epithelial sloughing. The second term is followed by the two migration terms: (1) where, when the value of [K− A(t)] is positive indicating adherent sites are available, the mucus-living population [M(t)] migrates to the epithelial cells at rate *a* and (2) where a proportion, δ, of the adherent population migrates to the mucus. The values for each of the parameters in the model are then estimated from experimental data in the literature. For those parameters for which no data are available, a mathematical study of varying values for those rates is performed (see below). A similar procedure is followed for all of the populations tracked in the model: mucus-living *H. pylori* [M(t)], nutrients [N(t)], effectors [E(t)], and, later in the model, the host response, [I(t)] (see Refs 15,16 for full model details).

Each of the rate constants for the processes outlined above can be varied in the model and the corresponding outcome predicted. In the analyses presented in Fig. 1b, an outcome of 'mutualism' is defined as the bacterial populations reaching steady state, whereas 'parasitism' (disease) is said to occur if the bacterial population grows to infinity.

When the parameter governing the ability of bacteria to adhere is changed to create a 'virtual' mutant (i.e. by setting '*a*' to 0), the model predicts that colonization will be lost (noted as C for clearance in Fig. 1b). Assuming that attachment does not produce changes in the default values of other parameters, the model predicts that adherence is central to *H. pylori* persistence, and that it is the adherent population that serves to sustain colonization by acting as a core population. It is also possible that the mucus population acts to replenish the adherent population and serves as a reservoir for transmission to new hosts.

Microorganism–host co-regulation

Our model can also be used to explore the hypothesis that both microorganism and host regulate elements of their interactions[15]. For example, effectors produced by *H. pylori*, such as urease and other factors that inhibit the effects of acid or its production, can alter the environment to the benefit of *H. pylori*. The bacteria could also downregulate secretion of host



(b)

Label	Parameter definition	Change in value from mutualism: Lower value	Higher value
μ_M	Rate *H. pylori* is lost owing to shedding of mucus layer	P	C
μ_A	Rate *H. pylori* is lost owing to sloughing of epithelial cells	P	C
g_M	Proportionate amount of nutrient utilized by mucus *H. pylori*	M	M
g_A	Proportionate amount of nutrient utilized by adherent *H. pylori*	M	M
K	Carrying capacity of the epithelium	M	P
a	Rate (proportion) that mucus *H. pylori* migrates to adherent *H. pylori*	C	M
r	Growth rate of *H. pylori* population	C	P
δ	Migration rate of adherent *H. pylori* to mucus *H. pylori*	C	M
b	Ratio for immune response inhibition of effectors	M	M
c	Maximal production of effectors by *H. pylori*	C	P
β	Proportion of nutrients produced based on effectors	C	P
τ	Rate effectors are depleted by epithelial cell interaction	M	M
η	Monitors production of effectors proportional to nutrients	P	C
k_1	Growth rate of host response	high M	low M
k_2	Maximal capacity of host response	P	C
k_3	Adherent/mucus influence on host response	M	M

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Fig. 1. Theoretical model of the relationship between *Helicobacter pylori* and its host, together with a study of parameter variations on *H. pylori* colonization. **(a)** presents the model showing interactions and rate parameters, and **(b)** gives the definition of parameters and predicted outcomes of changes in parameter values from steady-state conditions. The model predicts that altering the values of several of the parameters leads to clearance (C, in which *H. pylori* populations die out), mutualism (M, in which *H. pylori* persists in steady state), or parasitism (P, in which *H. pylori* density is so high that it promotes the risk of disease). Note that disease is not always characterized by a high bacterial load, and even low numbers of colonizing bacteria in the appropriate host could promote disease.

effector molecules in a way that could be adaptive for maintenance of their niche, for example, as part of an anti-inflammatory mechanism. The latter response could also be beneficial to hosts in which colonization is persistent. Such a co-regulated system could be partly responsible for distinct outcomes of colonization,

because all hosts are unique, as are their bacterial populations. To illustrate this point, two key parameters that are not measurable experimentally at present can be used to explore the impact of elements of the co-regulated system. One parameter is the production of effectors by *H. pylori* ('c' in Fig. 1b). This parameter can be considered to represent the ability of *H. pylori* to monitor available nutrients and to effect increases, via the host. Another parameter is the creation of nutrients in the host as a result of the production of effector molecules by *H. pylori* (parameter 'β'). This parameter represents the responsiveness of individual hosts (and host micro-niches) to bacterial effectors. The predicted outcome of lowering or increasing the values of these parameters on mutualism (persistence) or parasitism (disease) is presented in Fig. 1b.

Competition

Competition can also be explored within the co-regulation framework[15,16]. If two strains of *H. pylori* are competing for adherence receptors/sites, and one is slightly more motile or more efficient at adhering than the other, then this strain will out-compete the other over either a short or long time frame, depending upon the extent of the differences between strains. Modeling indicates that any two strains will never co-exist indefinitely in a given niche if they differ in any way – one will always have a competitive advantage. The presence of more than one *H. pylori* strain in a gastric biopsy could indicate a short-term phenomenon before one strain has out-competed the other, or it could indicate the existence of multiple micro-niches.

To identify the early dynamics that occur at the time of *H. pylori* colonization, our model was developed to incorporate the role of a generalized host response. (Future models will explore the specific immune response to *H. pylori*.) Although there are humoral and cellular immune responses to *H. pylori*, both appear ineffective in clearing the bacteria and preventing colonization in most individuals. However, transient colonization has been documented in both humans and experimentally challenged animals[24], indicating that such host responses can be effective under particular circumstances. The theoretical model in Fig. 1a incorporates three key elements of a generalized host response: (1) the growth rate of the host response (k_1); (2) the maximum allowable host response (k_2); and (3) the relative influences of adherent and free-living (mucus) *H. pylori* on the host response (k_3)[16]. The model indicates that k_2, the maximal host capacity for the response to the organism, is a critical parameter in determining the outcome of colonization (Fig. 1b). In the case of co-infection with HIV and *H. pylori*, clinical studies indicate that mutualism prevails[25]. Thus, the host response could indeed represent factors other than an adaptive immune response, as the immunosuppression associated with AIDS does not lead to unlimited bacterial growth.

In summary, the host-response model permits examination of both the early events following acquisition of *H. pylori* and the development of colonization. As indicated in Fig. 1b, this model can be used to begin to predict whether perturbations in host parameters/responses lead to persistence or loss of the organism.

Experimental tests in transgenic mice

The development of mathematical models can guide and test intuition about a biological system. The challenge presented by modeling is to devise direct tests of the roles of proposed host and/or microbial factors using informative and relevant biological assay systems. Here, we focus on two transgenic mouse models that have tested the notion that the location of a microorganism in the continuum between mutualism and parasitism is influenced by its ability to produce adhesins recognized by gastric epithelial receptors, and by the nature of the epithelial cell target.

Several *H. pylori* gene products have been postulated to participate in adhesive interactions with host gastric epithelial receptors. Bóren and co-workers demonstrated that BabA, a protein with homology to the outer membrane protein (Omp) superfamily, binds to the human histo-blood group antigen Lewis^b (Fucα1,2Galβ1,3[Fucα1,4]Glc*N*Acβ)[26]. One transgenic mouse model has examined the effects of *in vivo* binding of *H. pylori* to Lewis^b glycans produced by members of the pit cell lineage. Some clinical isolates of *H. pylori* can bind to sialylated glycans, including those that are expressed in gastric-adenocarcinoma-derived cell lines[27,28]. A second transgenic model has investigated the effects of bacterial binding to NeuAcα2,3Galβ1,4 glycans produced by gastric epithelial lineage progenitors that are amplified as a result of a genetically engineered ablation of parietal cells.

Engineering production of Lewis^b glycans

The glandular epithelium of the adult mouse stomach contains thousands of tubular-shaped mucosal invaginations known as gastric units. Epithelial renewal within these units occurs continuously, throughout the life of the animal. Lineage-tracing studies indicate that each of the stomach's epithelial cell types is derived from a common multipotent stem cell[29–34]. One of the stem cell's committed daughters gives rise to mucus-producing pit cells, which complete their terminal differentiation during a rapid, upward migration from the stem cell zone ('isthmus') located in the middle of each gastric unit, to the apical orifice of each unit. Once mature pit cells arrive at the surface epithelium, they are removed by exfoliation into the lumen, or are phagocytosed by their epithelial neighbors. The pit cell lineage is renewed every 3 d in adult mice[31].

Approximately 70% of humans synthesize Lewis^b-containing glycans[19]. This di-fucosylated structure is limited to pit cells within the gastric epithelium[19]. The FVB/N strain of mice synthesizes H-type-1-like epitopes (Fucα1,2Galβ1,3[Fucα1,4]Glc*N*Acβ) in pit cells, but not Lewis^b itself[35]. However H-type 1 structures can serve as acceptors (substrates) for the human Lewis enzyme, α1,3/4 fucosyltransferase (α1,3/4 FT), in the transglycosylation process that leads to Lewis^b formation. Expression of human α1,3/4 FT in the pit cell lineage of FVB/N transgenic mice results in the

production of Lewis^b-containing glycoproteins from the first week of postnatal life through at least the next 2 years[35,36].

Genetically engineered pit cell Lewis^b glycans function as receptors for adhesins produced by clinical isolates of *H. pylori*[36]. *In vivo* studies of the effects of binding to Lewis^b receptors have been performed with an isolate recovered from a Lewis^b-positive Peruvian patient with gastritis[36]. This isolate, designated Hp1, contains the first seven genes of the *cag* PAI [i.e. open reading frames (ORFs) HP0520–527 (Ref. 37) are present and HP0528–HP0547 are absent] (A. Sillén *et al.*, unpublished). Hp1 colonizes the stomachs of 4–52-week old α1,3/4 FT mice and their age-matched non-transgenic littermates. The efficiency and density of colonization are comparable in both groups when assessed 4, 8, 16 or 32 weeks after introduction of bacteria (Refs 36,38; J. Guruge *et al.*, unpublished). However, in the transgenic mice, bacteria are attached to Lewis^b-positive pit cells and are associated with the luminal mucus whereas in normal littermates, bacteria appear to be restricted to the luminal mucus[36,38].

How can we understand the persistence of infection in non-transgenic littermates where attachment to the epithelium is not evident, given the results of the modeling experiments? This question emphasizes the caution that must be exercised when extrapolating directly from modeling with a defined set of input parameter values to an *in vivo* experiment. Our model was designed to study the equilibrium reached between a host and a bacterial population. Increasing one parameter might result in decreases in another. What affords the adherent bacterial population the power to sustain colonization is a given set of microbial characteristics. The adherent population enjoys a slower washout rate owing to the longer lifespan of epithelial cells compared with mucus gel. However, if the non-adherent population could adapt itself or its environment to slow its washout (e.g. by delaying gastric emptying[39]), then it might be able to sustain itself.

The distinct distribution of bacteria in the stomachs of normal and transgenic mice, coupled with the similarities in their density of colonization, makes it possible to attribute differences in host responses to the effects of attachment. *In vivo* binding is associated with more-severe gastritis. Eight weeks after colonization of 6-week-old animals, the number of CD45$^+$ cells is ~fivefold greater in the gastric epithelium of transgenic compared with non-transgenic littermates[38]. In both groups, CD4$^+$, TCRαβ$^+$ cells predominate, as in humans who have *H. pylori*-induced chronic gastritis[40].

H. pylori attachment to Lewis^b-positive pit cells elicits production of antibodies that react with both bacterial lipopolysaccharide (LPS)- and host parietal-cell-associated Lewis^x immunodeterminants. Most clinical isolates of *H. pylori* contain Lewis^x (Galβ1,4 [Fucα1,3]Glc*N*Acβ) and Lewis^y (Fucα1,2Galβ1,4 [Fucα1,3]Glc*N*Acβ) epitopes in their LPS (Ref. 41). These epitopes are produced by the microorganism's own α1,2- and α1,3- FTs[37]. Synthesis is subject to phase variation[42,43], and although the direct role of Lewis expression remains unclear, this phase variation could provide a pool of bacterial phenotypes that facilitates selection of host-adapted populations. In uninfected normal and α1,3/4 FT transgenic FVB/N mice, Lewis^x immunodeterminants are also produced by a subset of acid-producing parietal cells; production is unaffected by *H. pylori*[36,38]. More than 80% of infected transgenic and <15% of infected normal littermates develop an antibody response to Lewis^x epitopes 8 weeks after initial colonization[36,38]. The titer of Lewis^x antibodies is significantly greater in α1,3/4 FT mice compared with non-transgenic controls (both groups were matched for age, gender and density of *H. pylori* infection). Moreover, high antibody titers are associated with greater parietal cell loss[36].

Although the relative contributions of cytotoxic T cells and Lewis^x antibodies to this parietal cell loss have not been defined, these results suggest the following hypothesis. A host harboring bacteria that express adhesins which recognize a gastric epithelial receptor has a higher risk of developing more-severe gastritis. Furthermore, if the colonizing strain shares immunodeterminants with the host (e.g. Lewis^x), the destiny of infection could be skewed towards cross-reactive immunopathology, including parietal cell loss. The idea that structural similarities between microbial epitopes and epitopes normally expressed in host cell lineages can, under certain circumstances, lead to self-directed immunity is an emerging theme in microbial pathogenesis.

The effects of parietal cell ablation

Genetically well defined and manipulatable animal models are needed to dissect the molecular mechanisms that underlie a postulated sequence of *H. pylori*-associated changes in infected hosts, which progresses from superficial gastritis to chronic atrophic gastritis (including parietal cell loss), to dysplasia/metaplasia, and finally to adenocarcinoma[44]. Wang and co-workers found that normal C57Bl/6 mice infected with *Helicobacter felis* develop atrophic gastritis as well as intestinal metaplasia[45]. Moreover, in transgenic mice that overexpress the trophic hormone gastrin under the control of transcriptional regulatory elements from the rat insulin I gene, long-term infection with *H. felis* is associated with progression to frank neoplasia[46]. Wang *et al.* proposed that persistent hypergastrinemia and persistent *H. felis* infection are synergistic risk factors for development of parietal cell loss with progression to gastric cancer[46]. *H. pylori* was not examined in these mice.

A recent transgenic mouse model has provided some insights into the mechanisms that could affect initiation or progression of neoplasia in humans who lose parietal cells during the course of persistent *H. pylori* colonization[38]. When parietal-cell-specific transcriptional regulatory elements from the non-catalytic β subunit gene of mouse H$^+$/K$^+$ ATPase were used to direct expression of an attenuated diphtheria

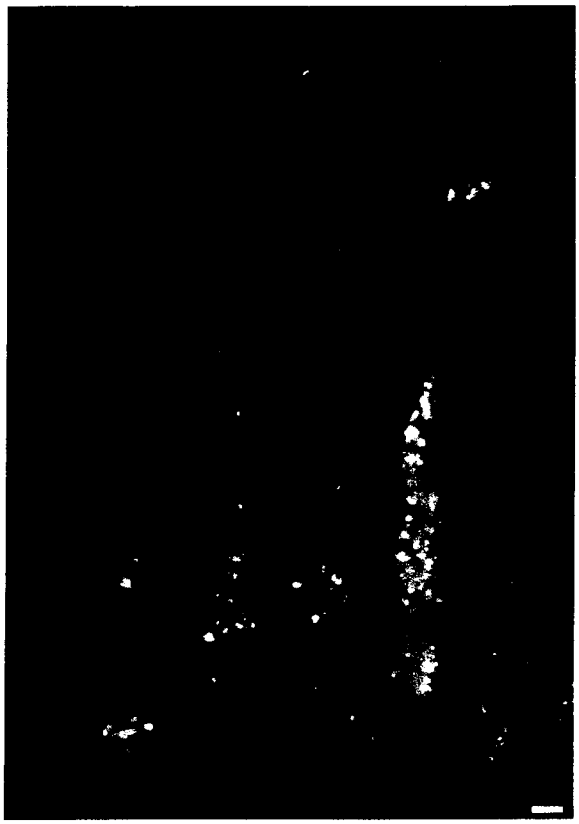

Fig. 2. *Helicobacter pylori* expresses adhesins that bind to Lewis^b glycan receptors produced in the pit cell lineage and to NeuAcα2,3Galβ1,4Glc-containing glycan receptors produced by gastric epithelial progenitors. Shown is a multi-label immunohistochemical study of a section from the corpus region of a bi-transgenic mouse obtained from crossing α1,3/4 fucosyltransferase (FT) mice and *tox*176 mice. The 14-week-old bi-transgenic mouse lacks parietal cells. It expresses Lewis^b glycans in its pit cells (visualized as blue using monoclonal antibodies to Lewis^b), and NeuAcα2,3Galβ1,4Glc glycans in its expanded epithelial progenitor cell population (detected as green using a lectin – *Maackia amurensis* agglutinin). Bacteria (detected as red using antibodies to surface proteins) are shown binding to both populations of cells. Scale bar = 25 μm.

toxin fragment A (*tox*176), parietal cells were eliminated from gastric units[38]. This loss of parietal cells produced a block in the differentiation of neck to zymogenic (chief) cells, plus enhanced proliferation of the presumptive multipotent stem cell and its immediate committed daughters. Over time, these lineage progenitors increased in number until they became a prominent and predominant population in gastric units[38].

The histopathological features present in the stomachs of *tox*176 mice resemble those encountered in patients with chronic atrophic gastritis, namely, loss of parietal and zymogenic cells and increased epithelial proliferation. *tox*176 mice provide at least one potential mechanistic explanation for why patients with chronic atrophic gastritis appear to be at risk for the development of gastric adenocarcinoma: parietal cell loss provides an opportunity for *H. pylori* to interact with an expanded progenitor population bearing a sialylated glycan receptor for one or more of its adhesins. Gastric progenitor cells express NeuAcα2,3Galβ1,4 glycans, which can be detected with *Maackia amurensis* agglutinin (MAA)[38]. Amplification of lineage progenitors in adult conventionally raised or germ-free *tox*176 mice is accompanied by increases in the census of MAA-positive cells[38]. *In situ* binding studies using stomach sections prepared from uninfected α1,3/4 FT and *tox*176 mice indicate that clinical isolates such as Hp1 constitutively express at least two functional classes of adhesins – one that recognizes Lewis^b and another that interacts with NeuAcα2,3Galβ1,4-containing glycoconjugates (Fig. 2).

Like Lewis^b, NeuAcα2,3Galβ1,4 epitopes produced in *tox176* mice serve as receptors for *H. pylori* adhesins *in vivo*. Comparisons of normal, α1,3/4 FT, and *tox176* mice that received a single inoculation of 10^7 colony-forming units (CFU) of Hp1 revealed that all groups of animals became colonized (i.e. acid production is not a requirement). An 8-week infection of *tox176* mice produces a 15-fold greater number of mucosal CD45$^+$ lymphocytes compared with age-, gender- and microbial-density-matched normal littermates. [The number of CD45$^+$ cells is too low to be quantified by fluorescence-activated cell sorting (FACS) in uninfected, age-matched *tox176* and normal mice[38]]. The enhanced cellular immune response associated with binding to lineage progenitors consists predominantly of CD4$^+$ TCRαβ$^+$ lymphocytes (as in α1,3/4 FT mice). *tox*176 mice also exhibit an increased occurrence and titer of Lewis^x antibodies[38]. Because *tox*176 animals lack Lewis^x-containing parietal cells, this humoral response must be elicited by bacterial-LPS-associated rather than host-parietal-cell-associated Lewis^x immunodeterminants.

Together, these results emphasize that a combination of factors could determine the evolution of *H. pylori* colonization towards parasitism (Fig. 3). When the relationship between host and microorganism results in loss of parietal cells, as in chronic atrophic gastritis, bacterial tropism to lineage progenitors can occur if there is a matching of microbial adhesin and host receptor production. Binding of *H. pylori* to this population of epithelial cells with a high proliferative potential and a relatively long residence time in the gastric ecosystem could put the host at considerably higher risk of initiation of neoplasia than could *H. pylori* binding to a terminally differentiated pit cell.

These findings unite several previous observations. First, clinical isolates of *H. pylori* have been reported to bind to NeuAcα2,3Galβ1,4 glycans produced by several human gastrointestinal adenocarcinoma-derived cell lines (e.g. Ref. 28). The *tox*176 transgenic mouse model establishes that these sialylated epitopes function as adhesin receptors *in vivo*. Second, normal human gastric units contain rare isthmal cells that produce NeuAcα2,3Galβ1,4 epitopes. Parietal cell loss in humans with chronic atrophic gastritis is associated with augmented production of these NeuAcα2,3Galβ1,4-containing glycans. Extrapolating from the *tox*176 mouse results, it is possible that increased proliferation of lineage progenitors owing

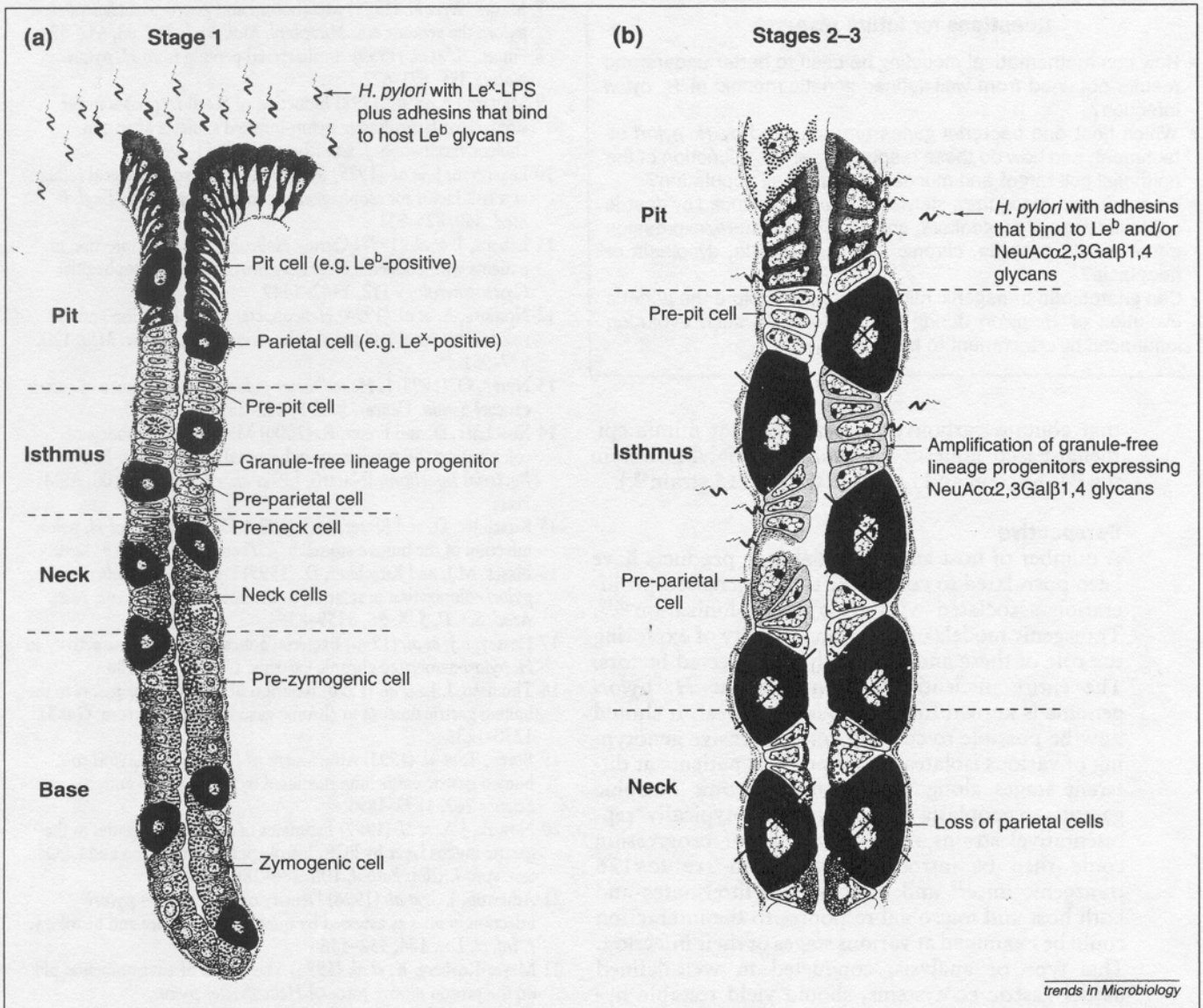

Fig. 3. Proposed combinatorial model of *Helicobacter pylori* infection that could result in progression to chronic atrophic gastritis and initiation of neoplasia. **(a)** Stage 1. A colonizing strain expresses an adhesin that allows it to bind to a terminally differentiated gastric epithelial cell lineage in its host (e.g. pit cells). Mathematical modeling studies presented in Fig. 1b indicate that adherence is central to *H. pylori* persistence. Adherence also promotes host recognition of microbial antigenic determinants through mechanisms that remain to be defined. If the colonizing strain also expresses an epitope on its surface [e.g. Lewis^x (Le^x)] that is shared by the host parietal cells, then the host could be at an increased risk for developing crossreactive immunopathology and parietal cell loss. **(b)** Stage 2. Loss of parietal cells promotes expansion of isthmal lineage progenitors. In some or all hosts, these progenitors express sialylated oncofetal epitopes (general structure = NeuAcα2,3Galβ1,4). If the colonizing strain is able to express adhesins that recognize these sialylated glycans, it can attach to the progenitors. Expression of different classes of adhesins favors persistence in a changing host gastric ecosystem. Stage 3. Depending upon the genotype of the colonizing strain, the genotype of the host, and/or unspecified environmental factors, attachment to lineage progenitors results in augmentation of proliferation, acquisition of mutations in the target cell population, and initiation of neoplasia. Immune cell populations might contribute to initiation of tumorigenesis through production of factors that affect epithelial proliferation, apoptosis or migration. The schematic representations of gastric units in this figure are modified, with permission, from Ref. 30.

to parietal cell loss could underlie the observed expansion of NeuAcα2,3Galβ1,4-producing cells in patients with chronic atrophic gastritis. Third, NeuAcα2,3Galβ1,4 glycans are expressed in gastric adenocarcinomas as well as in pre-cancerous states that have been associated with persistent infection[47]. These sialylated glycans could function as more than just markers of tumorigenesis, and rather as mediators.

A central challenge to *H. pylori* is to adapt to changes in the gastric ecosystem that could be partly a result of its own colonization. If a colonizing strain can express multiple different adhesins with different receptor/cellular specificities, then this strain should be better equipped to persist in the stomach in the face of a changing epithelial cell milieu. More information is needed about whether *H. pylori* can adapt its pattern of adhesin production *in vivo* to coincide with the available epithelial receptor milieu, or whether it can directly regulate the pattern of expression of adhesin receptors in the epithelial cells of its host. (It is interesting to note that some clinical isolates from symptomatic patients have LPS structures

Questions for future research

- How can mathematical modeling be used to better understand results obtained from well-defined genetic models of *H. pylori* infection?
- Which host and bacterial genes are regulated by *H. pylori* attachment, and how do these responses vary as a function of the epithelial cell target and mucosal immune cell population?
- What adhesin recognizes sialylated glycans produced by gastric epithelial lineage progenitors, and does its structure/expression differ in mild gastritis, chronic atrophic gastritis, dysplasia or neoplasia?
- Can gnotobiotic transgenic mice be used to explore the genetic evolution of *H. pylori* during infection and is such evolution influenced by attachment to host cells?

that contain carbohydrate epitopes that mimic epithelial-based adhesin receptors: i.e. sialyl-Lewis^x in the P466 strain and Lewis^b in the UA915 strain[48].)

Perspective

A number of host and microbial gene products have been postulated to contribute to the increased proliferation associated with *H. pylori* colonization[49,50]. Transgenic models offer the opportunity of exploring the role of these and previously unsuspected factors. The entire nucleotide sequence of the *H. pylori* genome is known for two clinical isolates[6]. It should now be possible to conduct comprehensive genotyping of various isolates recovered from patients at different stages along the proposed chronic atrophic gastritis to neoplasia progression. Genotypically 'representative' strains from each stage of progression could then be introduced into germ-free *tox*176 transgenic mice[38] and their normal littermates and both host and microbial responses to the interaction could be examined at various stages of their infection. This type of analysis, conducted in well-defined model gastric ecosystems, should yield testable hypotheses about microbial and host determinants of mutualistic vs parasitic relationships.

Authors' note

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