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Competing interests statement
The author declares no competing financial interests.

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OPINION

Helicobacter pylori phase variation, immune modulation and gastric autoimmunity

Mathijs Bergman, Gianfranco Del Prete, Yvette van Kooyk and Ben Appelmelk

Abstract | *Helicobacter pylori* can be regarded as a model pathogen for studying persistent colonization of humans. Phase-variable expression of Lewis blood-group antigens by *H. pylori* allows this microorganism to modulate the host T-helper-1-cell versus T-helper-2-cell response. We describe a model in which interactions between host lectins and pathogen carbohydrates facilitate asymptomatic persistence of *H. pylori*. This delicate balance, favourable for both the pathogen and the host, could lead to gastric autoimmunity in genetically susceptible individuals.

Helicobacter pylori is a human pathogen that persistently colonizes the stomach of approximately half of the world's population, for as long as the lifetime of its host. Colonization of the gastric mucosa typically occurs during childhood, and ~10% of those infected with *H. pylori* ultimately develop disease, which ranges from gastritis to peptic-ulcer disease to mucosa-associated lymphoid tissue (MALT) lymphoma or gastric cancer[1]. However, in most cases, *H. pylori* persists without inducing clinical disease in its host, indicating that, at the gastric mucosal interface, there is a host–pathogen equilibrium that is beneficial for both organisms. Indeed, more recent data indicate that, in a small subpopulation of infected individuals, infection with *H. pylori* during childhood could protect against the later development of severe gastric-reflux disease, Barrett's oesophagus and adenocarcinoma of the oesophagus[2].

H. pylori is equipped with an impressive range of mechanisms that facilitate persistent colonization of its host[3], and because of the severity of *H. pylori*-associated diseases, the virulence factors of *H. pylori* have been studied extensively[4]. Infection with *H. pylori* results in vigorous innate and acquired immune responses by the host, as manifested by release of cytokines by epithelial cells and infiltration of the gastric mucosa by neutrophils, macrophages and lymphocytes, as well as by induction of specific humoral responses[5–10]. *H. pylori* triggers the innate immune system through interaction with Toll-like receptor 2 (TLR2)[11] and increases the influx of neutrophils and mononuclear cells to the mucosa through expression of neutrophil-activating protein[12]. In addition, *H. pylori* uses several mechanisms to evade or downregulate both innate and adaptive host immune responses. Lipopolysaccharide (LPS, see Glossary) expressed by *H. pylori* has low endotoxic and immunobiological activity compared with LPS of other bacteria[13,14] and can antagonize TLR4 signalling[11]. In addition, *H. pylori* can evade interaction with the host receptor TLR5 (REF. 15), a property that could contribute to its persistence at the mucosal surface. Arginase expressed by *H. pylori* downregulates nitric-oxide production by macrophages[16]. Efficient phagocytosis and killing of *H. pylori* is prevented by the presence of the *cag* pathogenicity island, which encodes a type IV secretion system[17,18]. Finally, *H. pylori* vacuolating cytotoxin A (VacA) inhibits the activation and function of T cells[19,20].

Helicobacter spp. are proposed to be native inhabitants of the stomach, and substantial evidence supports the idea of co-evolution of *H. pylori* and humans[21]. It is thought that, during this proposed co-evolution, bacteria were selected for their ability to induce sufficient epithelial damage to free nutrients but not to threaten the viability of the host. Insight into the host–pathogen interactions that are involved in *H. pylori* persistence should increase our understanding of molecular mechanisms that are involved in persistence of other, less well-known, human pathogens. In this article, we focus on a recently discovered role for *H. pylori* LPS in the modulation of the host immune response towards a local inflammatory environment that facilitates persistence. Following initial colonization by *H. pylori*, most infected individuals remain asymptomatic for decades; however, a small number of individuals can develop full-blown gastric autoimmunity as a result of persistence of this microorganism.

Host responses to *H. pylori* infection

Cytokines, such as interleukins and other lymphokines, are regulatory proteins that are released by cells of the immune system and function as intercellular mediators to initiate and coordinate immune responses (FIG. 1). $CD4^+$ T cells can be categorized according to their cytokine-secretion profile and cytotoxic potential. There are two main subsets of T helper (T_H) cells. T_H1 cells secrete tumour-necrosis factor (TNF) and interferon-γ (IFN-γ), lyse antigen-loaded target cells through mechanisms that are mediated by perforin or FAS (also known as CD95), and are involved in the cellular branch of defence against intracellular pathogens. T_H2 cells secrete interleukin-4 (IL-4), IL-5 and IL-10, are involved in downregulation of T_H1-cell-mediated inflammatory events and facilitate production of antibodies by B cells.

The outcome of bacterial infections is determined by bacterial virulence factors, which can differ between strains, as well as by host genetics, in particular the immune-response genes. Following experimental infection of mice with *H. pylori*, colonization of the stomach depends on the genetic background of the mouse strain used[22–25]. In addition, when rhesus monkeys were infected with a mixture of several *H. pylori* strains, different *H. pylori* strains from the inoculum were selected for initial colonization in individual monkeys, illustrating the susceptibility of an individual host for a particular *H. pylori* strain[26].

Differences in susceptibility of mouse strains to colonization with *H. pylori* and the associated pathology have mainly been attributed to the differences in cytokine production on infection, and the absence of gastric inflammation following infection of mice with *H. pylori* is associated with production of the anti-inflammatory cytokine IL-10 (REF. 27). However, the role of pro-inflammatory cytokines in the infected gastric mucosa is unclear. Whereas IFN-γ is an important mediator of inflammatory damage of the mucosa[28], IFN-γ-mediated inflammation also seems to protect the host from colonization with *H. pylori*[24,29]. For vaccine development, polarization of the host immune response towards a T_H2-cell response has been proposed to be the key element that is required to achieve protection against *H. pylori*[12]. However, in the light of recent findings, this idea might be only partly correct. IL-12 — a cytokine that is important for the development of T_H cells into T_H1 cells — increases *H. pylori* colonization of the stomach of C57BL/6 mice (which are 'T_H1 prone': that is, owing to their genetic background, they are predisposed to produce mainly T_H1 cytokines following bacterial infection), but it seems to protect BALB/c mice (which are 'T_H2 prone') from colonization[25]. Together, these observations indicate that a particular balance between T_H1 and T_H2 cells, possibly influenced by overall genetic composition of the host, is required for persistent colonization following initial infection with *H. pylori*.

Development of *H. pylori*-associated pathology is associated with the ratio of pro- and anti-inflammatory cytokines, which is indirectly or directly influenced by the genetic make-up of the host. For humans, in the presence of *H. pylori*, genetic polymorphisms in the IL-1 gene cluster that are suspected of increasing production of IL-1β (that is, polymorphisms in *IL1B*, which encodes IL-1β, and *IL1RN*, which encodes the receptor antagonist of IL-1β), increase the risk of gastric cancer and its precursors, hypochlorhydria and atrophic gastritis[30,31]. In addition to IL-1 gene-cluster polymorphisms, pro-inflammatory genotypes of *TNF* and *IL10* (that is, polymorphisms in *TNF* and *IL10* that are associated with decreased production of cytokine) have been identified as risk factors for gastric cancer[32]. An increasing number of pro-inflammatory genotypes (*IL1B*-511T, *IL1RN**2*2*, *TNF*-308A and *IL10* ATA/ATA (homozygous for *IL10*-1082A, *IL10*-819T and *IL10*-592A)) seems to progressively increase the risk of gastric cancer. When three to four of these polymorphisms are present, the risk of gastric cancer is 27-fold higher than when none is present[32]. In conclusion, genes that encode immune-response regulators (that is, IL-1, IL-12 and TNF) or cytokines secreted by T_H-cell subsets (that is, IL-4 and IL-10) are implicated in susceptibility to development of *H. pylori*-associated diseases.

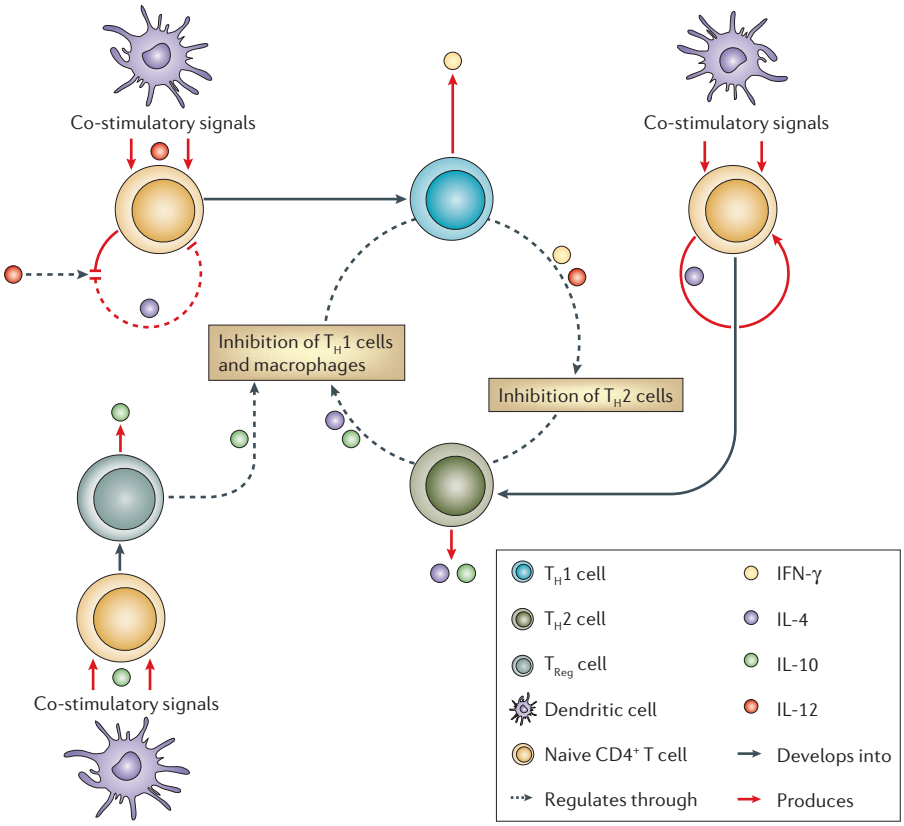

Figure 1 | **Schematic representation of the induction and cross-regulation of the development of T_H cells into T_H1 and T_H2 cells.** Dendritic cells that are involved in antigen presentation provide naive T cells with several co-stimulatory signals and either interleukin-12 (IL-12) or no IL-12. In the presence of IL-12, the autocrine production of IL-4 by naive $CD4^+$ T cells is inhibited, and IL-12 drives the differentiation of these T cells into effector T helper 1 (T_H1) cells, which produce interferon-γ (IFN-γ) and activate macrophage functions. In this model of T-cell activation, IL-12 inhibits the development of concomitant T_H2-cell responses. In the absence of IL-12, naive $CD4^+$ T cells are driven by autologous IL-4 to develop into effector T_H2 cells, which induce B-cell differentiation and inhibit macrophage functions. Through the delivery of co-stimulatory signals and inhibitory cytokines (such as IL-10), dendritic cells activate $CD4^+CD25^+$ regulatory T (T_{Reg}) cells that downmodulate T-cell and macrophage responses through different mechanisms that involve both soluble and membrane-bound signals.

Because *H. pylori*-associated diseases can take decades to develop, data on cytokine responses in the gastric mucosa during acute *H. pylori* infection are largely derived from animal studies. In rhesus monkeys, acute *H. pylori* infection induces a response in which T_H1 cells predominate[33], which is concordant with the T_H1-cell response that is found in association with gastric pathology in *H. pylori*-infected humans[34,35]. Peptic ulceration is associated with *H. pylori*-specific, local, gastric T_H1-cell responses. By contrast, in patients with asymptomatic chronic gastritis — accounting for the 80–90% of individuals who are infected but do not develop overt disease — most *H. pylori*-specific gastric T cells are T_H0 cells, which secrete both T_H1 and T_H2 cytokines[36] (see Supplementary information S1,S2 (figure and table)). So, data obtained from humans indicate that *H. pylori*-infected individuals who can overcome the initial T_H1-cell-dominated response that occurs on infection, and can mount a mixed T_H1- and T_H2-cell response to *H. pylori* in their gastric mucosa, could be persistently colonized by the microorganism without developing clinical disease.

The host genetic factors that are involved in the shift from an acute T_H1-cell response towards a mixed T_H1- and T_H2-cell response during chronic *H. pylori* infection are unknown at present. However, data indicate that an exhaustive T_H1-cell response in the infected stomach can result in destruction of mucosal tissue and subsequent loss of the specific niche of *H. pylori* through development of gastric atrophy[37,38]. Therefore, the ability to modulate or suppress vigorous T_H1-cell responses could give *H. pylori* a selective advantage with respect to the co-evolution of microorganism and host (alluded to earlier), as well as with respect to the persistent colonization of an individual host. Recently, the possibility of there being such a mechanism in *H. pylori*, involving phase variation and expression of Lewis blood-group antigens, has been described[39].

Phase variation and immune modulation

LPS is an important structural component of the outer membrane of Gram-negative bacteria. Depending on the presence and functional transcription of genes that encode glycosyltransferases (enzymes that transfer a specific sugar residue to its acceptor), many bacteria — including *Salmonella* spp., *Neisseria meningitidis*, *Haemophilus influenzae* and *Campylobacter jejuni* — can alter the carbohydrate structures of their LPS, thereby changing the external appearance of the microorganism as perceived by the host, in particular by the host immune system[40–42]. Most (80–90%) *H. pylori* strains display Lewis blood-group antigens on their LPS, and these are similar to the Lewis blood-group antigens that are expressed on the mucosal surface of the human stomach[43]. Expression of Lewis antigens varies within a single strain of *H. pylori* as a result of phase variation — the high-frequency (up to 0.5%) 'on–off' switching of genes involved in LPS biosynthesis — a process that drives strain diversification. The molecular mechanisms that underlie the random nature of phase-variable expression of Lewis antigens are well documented and involve slippage of DNA polymerase during replication of certain glycosyltransferase genes that contain polycytosine tracts[44]. In addition, low pH (as in the stomach) has been proposed to be an environmental condition that selects for variants with increased expression of Lewis x and Lewis y[45]. In rhesus monkeys, Lewis-antigen expression by *H. pylori* corresponds to the host Lewis-antigen phenotype, indicating that host-adaptive bacteria have been selected, but this is not the case in humans[46,47]. In addition, the role of Lewis antigens in the attachment of *H. pylori* to the gastric mucosa seems to be limited[48]. Finally, a few studies report a correlation between Lewis antigens, the degree of leukocyte infiltration[49] and pathogenesis in symptomatic *H. pylori* infection[50]. However, because Lewis-antigen expression within an *H. pylori* strain is phase variable, these studies could be describing the host response to a mixed population of Lewis-antigen-positive and Lewis-antigen-negative *H. pylori*, leaving the significance and biological roles of phase variation and Lewis-antigen expression unclear.

Recently, we described the interaction of phase variants of *H. pylori* with DC-SIGN, a C-type lectin that is a cell-surface receptor on dendritic cells (DCs) that captures and internalizes antigens[51]. This interaction depends on both the presence and the three-dimensional structure of fucose-containing carbohydrate structures on LPS. Binding of *H. pylori* to DC-SIGN blocks skewing of the naive (that is, not previously exposed to antigen) $CD4^+CD45RA^+$ T-cell population towards T_H1 cells, whereas non-DC-SIGN-binding *H. pylori* variants promote development into T_H1 cells[39].

Glossary

Blood-group antigens
Several carbohydrate structures that are found at the cell surface of erythrocytes. They are encoded by a genetic locus with a variable number of alleles (for example, A, B and O in the ABO system), expression of which determines a blood-grouping reaction with a specific antiserum. The Lewis blood-group antigens are closely related to the ABO blood-group antigens and are expressed, for example, on the gastric mucosa.

Lipopolysaccharide
(LPS). A cell-surface carbohydrate antigen that is characteristic of Gram-negative bacteria. It consists of a glycolipid anchor (lipid A), an oligosaccharide linker (core) and an outward-protruding sugar polymer (O antigen). In *Helicobacter pylori*, the O antigen displays Lewis blood-group structures such as Lewis x and Lewis y, as well as Lewis a and Lewis b.

Phase variation
A random 'on–off' switching of genes that alters the expression of antigens at the bacterial surface. This process generates heterogeneity in a bacterial cell population.

T helper cell
(T_H cell). A T cell (that is, a type of leukocyte; also known as a T lymphocyte) that has cell-surface antigen receptors that bind fragments of antigens displayed by MHC class II molecules, which are expressed at the surface of antigen-presenting cells. Activated T_H cells express cytokines and membrane-associated co-stimulatory molecules that help other immune cells (including B cells, T cells and macrophages) to deploy their specific functions. T_H cells can be divided into subsets according to their cytokine-secretion profiles.

T_H0 cell
(T helper 0 cell). A type of T_H cell that secretes a mixture of T_H1 and T_H2 cytokines. Naive, undifferentiated T_H cells also belong to this T_H-cell subset. Depending on the antigen that is recognized and the environmental factors that are present (for example, cytokines), naive T_H0 cells can differentiate into either T_H1 or T_H2 cells. The reciprocal regulatory activity of T_H1 and T_H2 cells is thought to be one of the key regulatory mechanisms in the maintenance of 'immunological homeostasis' during health, and a disturbed balance of T_H1 and T_H2 cells has often been observed during infectious and/or autoimmune diseases.

T_H1 cell
(T helper 1 cell). A type of activated T_H cell that promotes responses associated with the production of pro-inflammatory cytokines and chemokines, and delayed-type hypersensitivity reactions. T_H1 cells secrete interferon-γ and lymphotoxin, activate phagocytosis and nitric-oxide production by macrophages, promote the activity of cytotoxic T cells and downregulate the differentiation of T_H cells into T_H2 cells.

T_H2 cell
(T helper 2 cell). A type of activated T_H cell that participates in phagocytosis-independent responses and downregulates pro-inflammatory responses that are induced by T_H1 cells. T_H2 cells secrete interleukin-4 (IL-4), IL-5, IL-6 and IL-10. These cytokines lead to the following: the activation, proliferation and differentiation of B cells; the production of antibody by B cells; the activation and prolonged survival of mast cells and eosinophils; and the inactivation of several functions of macrophages.

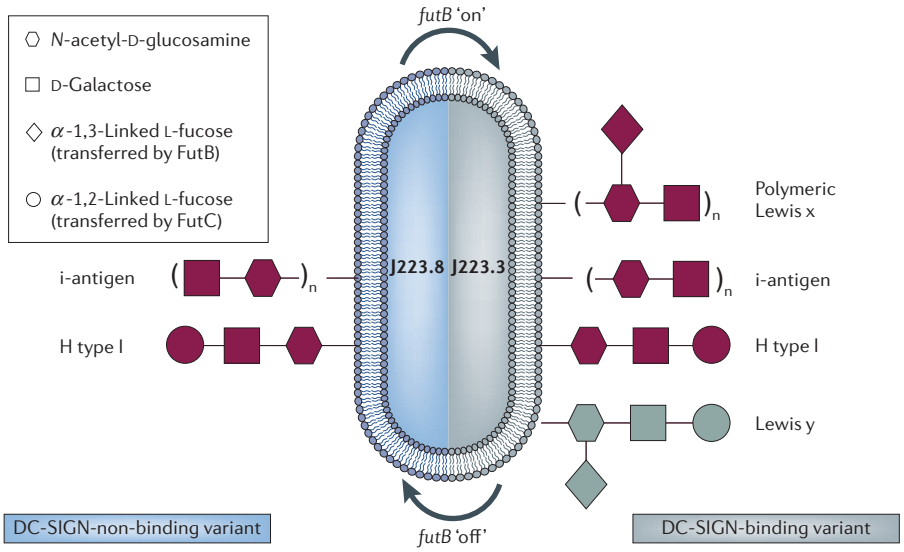

Figure 2 | **LPS phase variation generates a pool of DC-SIGN-binding and DC-SIGN-non-binding variants within a single strain of *Helicobacter pylori*.** Lipopolysaccharide (LPS) O-antigen expression in the *Helicobacter pylori* strain J223 is shown, as determined by binding profiles of specific monoclonal antibodies[39]. For visualization and simplicity, O antigens are depicted as distinct moieties in separate LPS molecules; however, several O antigens can be present in a single LPS molecule (for example, a chain of polymeric Lewis x, with a Lewis y structure at the end). The *H. pylori* phase variants J223.3 and J223.8 (which make up 20% and 80% of *H. pylori* strain J223, respectively) could be detected in a culture obtained directly from a gastric-biopsy specimen, after a minimal culture period and one passage on solid medium, showing that phase variation of *H. pylori* occurs in the stomach (that is, it occurs *in vivo*). Phase-variable expression of Lewis antigens by *H. pylori* strain J223 is caused by 'on–off' switching of the α-1,3-fucosyltransferase gene *futB*[39]. Whereas the α-1,3-fucosyltransferase gene *futA* is always switched off, the α-1,2-fucosyltransferase gene *futC* is always functionally transcribed (that is, switched on) in strain J223. When FutB is not expressed (as occurs in variant J223.8, shown in blue), the bacterium expresses LPS with O antigens that display carbohydrate moieties known as i-antigen and H type I, and it cannot bind DC-SIGN. By contrast, in variant J223.3, FutB is expressed, and this results in α-1,3-fucosylation of *N*-acetylglucosamine, adding polymeric Lewis x and, because of constitutive expression of FutC, Lewis y to the O-antigen carbohydrate-expression profile of J223.3 (shown in grey). J223.3 binds DC-SIGN through Lewis y. It should be noted that, whereas monomeric Lewis x confers binding to DC-SIGN, the capacity of polymeric Lewis x to bind DC-SIGN decreases with increasing number (n equals more than three) of repeating Lewis x moieties in the carbohydrate structure. Strain NCTC 11637 and variant 3a express polymeric Lewis x but do not bind DC-SIGN[39]. DC-SIGN-binding assays showed that DC-SIGN binding by *H. pylori* depends not only on the presence of fucosylated antigens (such as Lewis antigens) but also on the position of these fucosylated antigens in the LPS, and it is not conferred by Lewis-antigen expression *per se*[39,56].

HIV-1 and *Mycobacterium tuberculosis* interact with DC-SIGN through non-Lewis-antigen carbohydrate structures[52,53] (discussed later). Therefore, the process of LPS phase variation, which drives diversification of a single *H. pylori* strain into a pool of DC-SIGN-binding and DC-SIGN-non-binding (rather than Lewis-antigen-positive and Lewis-antigen-negative) phase variants (FIG. 2), could explain the success of *H. pylori* with regard to persistence in numerous hosts worldwide. We propose that a mixture of *H. pylori* variants that promotes a particular balance between T_H1 and T_H2 cells is optimal for persistent colonization of an individual, and this mixture depends on the genetic background of the host and is selected for by as-yet-unknown mechanisms. Bacterial phase variation, in combination with *H. pylori* virulence factors that have immune-modulatory activities, can then facilitate persistent colonization — as is observed in most people infected with this pathogen — and prevent development of severe atrophic gastritis and the subsequent loss of the ecological niche of *H. pylori*.

DC-SIGN binding facilitates persistence

Phase-variable expression of Lewis antigens by *H. pylori* — and the subsequent suppression of T_H1-cell responses, and protection of the host from excessive damage and atrophic gastritis, leading to loss of the ecological niche of *H. pylori* — could be regarded as a bacterial trait that is mutually beneficial for the pathogen and the host. The observation that most *H. pylori* strains express Lewis antigens supports the idea that the host might be able to positively select *H. pylori* strains that express Lewis antigens, by an as-yet-unknown mechanism(s). One mechanism could involve sampling of the *H. pylori* population by immature DCs that can protrude into the gastric epithelium[54] and selective binding to DC-SIGN by bacteria that express certain Lewis antigens. Following binding and phagocytosis of *H. pylori*, the DCs migrate to the gastric draining lymph nodes, where they fully mature, then present their processed antigens to T cells and coordinate the adaptive immune response[55] to *H. pylori*, including modulation of the balance of T_H1 and T_H2 cells. Therefore, constitutive uptake of Lewis-antigen-positive bacteria and subsequent antigen presentation by DCs could maintain the host immune response to *H. pylori*, creating 'micro-niches'[4] with a balance between T_H1 and T_H2 cells that renders these regions less 'hostile' to colonization.

Other human pathogens use their interaction with DC-SIGN to provide them with a competitive advantage. Binding of the HIV-1 envelope glycoprotein gp120 to DC-SIGN facilitates the transport of HIV-1 to lymph nodes, where efficient *trans*-infection of T cells occurs[52]. *M. tuberculosis* suppresses DC-mediated immune responses by binding DC-SIGN through mannose-capped lipoarabinomannan[53]. In addition, the interaction of carbohydrate surface antigens of *Leishmania mexicana* and *Schistosoma mansoni* with DC-SIGN causes a shift towards a T_H2-cell response, which is crucial for the persistence of these pathogens[56–58]. These findings indicate that the ability of pathogens to bind DC-SIGN using a fucosylated or mannosylated carbohydrate structure[56], rather than the expression of one particular antigen, is central to DC-SIGN-mediated host–pathogen interactions and modulation of the immune response of the host in favour of persistent infection.

Persistent infection and autoimmunity

Persistent bacterial infections can lead to autoimmune responses in genetically susceptible individuals. A well-documented example is Lyme arthritis, which is caused by infection with the pathogen *Borrelia burgdorferi*[59]. In patients with particular alleles of human leukocyte antigen DR4 (HLA-DR4; a major histocompatibility complex (MHC) class II molecule), autoimmune chronic synovitis can follow Lyme arthritis. This process is driven by molecular mimicry between an immunodominant T-cell epitope of *B. burgdorferi*

outer-surface protein A (amino acids 165 to 173 of OspA, OspA$^{165–173}$) and human lymphocyte function-associated antigen 1 (amino acids 332 to 340 of the α-chain of LFA1), an adhesion molecule that is highly expressed on the surface of T cells in the synovia[60]. T cells that react to OspA$^{165–173}$ are concentrated in the joints of these patients[61].

In addition, in the case of *H. pylori*, data are accumulating that indicate that chronic infection can lead to — or accelerate — the development of gastric autoimmunity in genetically susceptible individuals (as outlined in detail in REF. 62). Although *H. pylori* is not invasive and usually resides in the antrum of the stomach (the lower part, adjacent to the pyloric sphincter), 20–30% of *H. pylori*-infected patients develop antibodies that are specific for the gastric proton pump, H^+,K^+-ATPase, which is located in parietal cells in the corpus (the upper part of the stomach, adjacent to the oesophagus)[63]. The presence of these antibodies is correlated with the severity of gastric inflammation, increased atrophy and apoptosis in the corpus mucosa. Also, *H. pylori*-infected patients with autoantibodies have histopathological and clinical features that are similar to those of autoimmune gastritis (AIG)[64].

Additional, indirect evidence indicating a role for *H. pylori* in gastric autoimmunity is provided by epidemiological and intervention studies. A substantial proportion of patients with pernicious anaemia, which results from AIG, are infected with *H. pylori* or were infected with *H. pylori* (before the bacteria were cleared by the development of atrophy)[37,38], and the histologically defined early stages of AIG can be successfully treated by eradication of *H. pylori*[65–67].

Recently, we provided direct evidence for a role of *H. pylori* in gastric autoimmunity[68]. In patients with AIG who are infected with *H. pylori*, a considerable proportion of T cells isolated from the gastric mucosa were shown to react with both purified H^+,K^+-ATPase and *H. pylori* lysate: that is, they were crossreactive. The H^+,K^+-ATPase epitope that was recognized by each of the crossreactive T cells was identified using a library of synthetic peptides. On the basis of three types of assay — sequence similarity to H^+,K^+-ATPase peptide, *in silico* prediction of antigen presentation, and functional assays — nine *H. pylori* proteins each containing a different crossreactive epitope were identified. In the presence of synthetic peptide representing an *H. pylori* epitope, crossreactive T cells expressed the cytotoxic and pro-apoptotic properties that were likely to be responsible for the destruction of parietal cells in patients with AIG[68,69]. The *H. pylori* proteins that contain the crossreactive T-cell epitopes do not belong to the group of known immunodominant proteins of *H. pylori* (that is, CagA, VacA and urease) but, instead, are products of 'housekeeping' genes[68]. Consistent with the report that the combination of HLA-DR2 and HLA-DR4 and the combination of HLA-DR4 and HLA-DR5 are significantly associated with an increased risk of pernicious anaemia[70] — the end-point of AIG — we observed that activation of T cells that are specific for H^+,K^+-ATPase and T cells that crossreact with *H. pylori* and H^+,K^+-ATPase is HLA-DR restricted[68,69]. As observed for *B. burgdorferi* infection and Lyme arthritis, HLA-DR alleles could be involved in the selection of a specific *H. pylori* epitope that is recognized by crossreactive T-cell clones in the stomach of a human host (FIG. 3).

Destruction of gastric glands in patients with AIG is mediated by H^+,K^+-ATPase-specific, cytotoxic T_H1 cells[69]. Approximately 3% of healthy humans harbour H^+,K^+-ATPase-specific autoantibodies[63], indicating the presence of H^+,K^+-ATPase-specific autoreactive T cells that have escaped negative selection in the thymus but are kept under control by $CD4^+CD25^+$ regulatory T cells[71,72].

We propose that the development of gastric autoimmunity in genetically susceptible individuals occurs as a result of interactions between *H. pylori* and the host immune system. When the ingested strain is 'compatible' with its host during the initial stage of colonization[26], interplay between the immune response of the host and the virulence factors of *H. pylori* skews the gastric T-cell response, which is already T_H1-cell prone by nature[34], towards a strong T_H1-cell phenotype (FIG. 4a). Owing to inflammatory damage of the mucosa as a result of the ongoing T_H1-cell response, infected individuals who carry genetic polymorphisms that are associated with increased production of pro-inflammatory cytokines[30–32], or other host susceptibility factors, develop a gastric ulcer and possibly, eventually, gastric cancer[35]. However, at a certain time point in the infection process, by as-yet-unknown mechanisms, most infected hosts can downregulate the T_H1-cell response and switch to a mixed T_H1- and T_H2-cell response specific for *H. pylori*, and this mixed response facilitates the persistence of *H. pylori* without severe symptoms[36]. The phase-variable expression

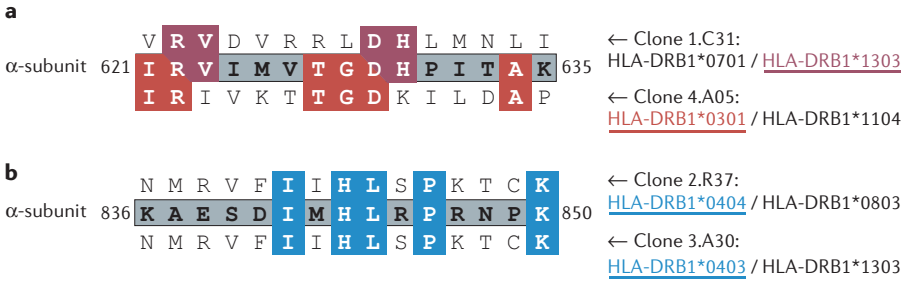

Figure 3 | **The HLA-DR alleles of an infected individual are involved in the recognition of crossreactive epitopes in the total pool of proteins expressed by *Helicobacter pylori*.** During presentation of a peptide antigen to a $CD4^+$ T cell, the peptide is located in the peptide-binding groove of a major histocompatibility complex (MHC) class II molecule. During *Helicobacter pylori* infection, human leukocyte antigen DR (HLA-DR) alleles have an important role through their ability to bind a particular sequence of amino acids (that is, a peptide). Presentation of *H. pylori*-derived peptides and subsequent activation of gastric T cells that are H^+,K^+-ATPase-and-*H. pylori*-crossreactive is HLA-DR restricted[68]. Therefore, the susceptibility of a person to develop gastric autoimmunity through molecular mimicry is likely to depend, in part, on his or her HLA haplotype. **a** | Crossreactive T-cell clones 1.C31 and 4.A05 recognize the same epitope of H^+,K^+-ATPase (amino acids 621 to 635 of the α-subunit; shown in grey). However, clone 1.C31 recognizes its specific peptide epitope in the context of an MHC class II complex that contains HLA-DRB1*1303, whereas clone 4.A05 requires HLA-DRB1*0301 for recognition of its epitope. As a consequence, clones 1.C31 and 4.A05 crossreact with peptides that are derived from different *H. pylori* proteins. (The non-coloured HLA allele indicated for each clone is the other HLA-DR allele that is present in the HLA haplotype of the individuals.) **b** | The crossreactive T-cell clones 2.R37 and 3.A30 both recognize an identical epitope of H^+,K^+-ATPase in the context of HLA-DRB1*04. As a consequence, these clones crossreact with an epitope in the same *H. pylori* protein. Identical amino-acid residues of H^+,K^+-ATPase and crossreactive *H. pylori*-derived peptide are boxed in the colour that is used to underline the HLA-DR allele required for antigen presentation. (For further details, see REF. 68.) So, an HLA-haplotype(s) that does not allow binding of any *H. pylori*-derived peptides similar to regions of gastric H^+,K^+-ATPase might protect an individual from development of gastric autoimmunity after infection with *H. pylori*.

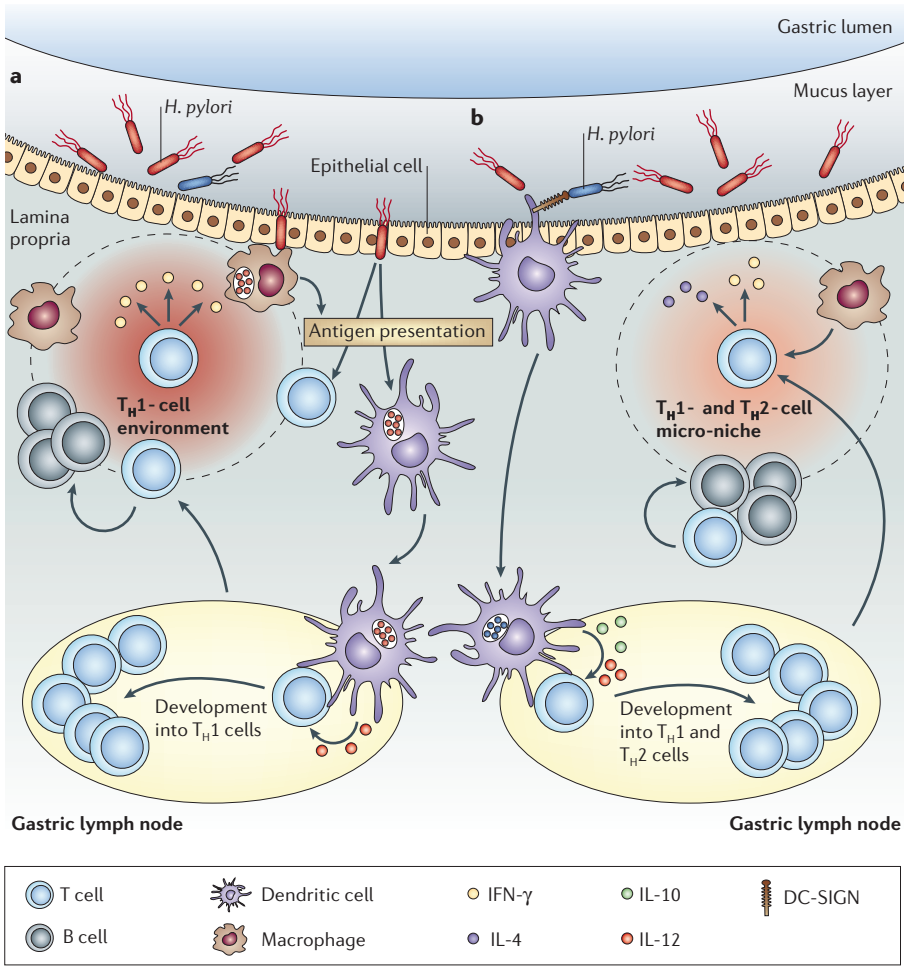

Figure 4 | ***Helicobacter pylori* phase variation suppresses development of T_H cells into T_H1 cells and could facilitate persistent colonization. a** | In susceptible hosts, *Helicobacter pylori* colonizes the stomach and induces upregulation of expression of MHC class II molecules and co-stimulatory molecules by epithelial cells, facilitating the presentation of *H. pylori* antigens by epithelial cells to gastric mucosal T cells[84,85], which are mainly of the T helper 1 (T_H1)-cell type (for further details, see main text). In addition, *H. pylori* antigens are presented by professional antigen-presenting cells such as macrophages and dendritic cells (DCs), which might acquire antigens as a result of epithelial-cell turnover. Development of *H. pylori*-associated peptic-ulcer disease is associated with the chronic predominance of effector T_H1 cells in the gastric mucosa[35]. **b** | In infected patients with asymptomatic chronic gastritis, *H. pylori*-specific T cells are mainly of the T_H0-cell type, which secrete both interferon-γ (IFN-γ) and interleukin-4 (IL-4). This indicates that most infected people switch from an acute gastric *H. pylori*-specific response that is mediated by T_H1 cells to a response that is mediated by T_H1 and T_H2 cells[36]. The mechanisms that are involved in the switch from a T_H1-cell response to a T_H1- and T_H2-cell response are unknown at present, but *H. pylori* phase variants that bind DC-SIGN to suppress the development of T_H cells into T_H1 cells, through IL-10 (REF. 39), might facilitate this switch and be selected for by the host. DC-SIGN-binding variants of *H. pylori* (blue) are selectively bound by DC-SIGN-expressing DCs that protrude from the gastric epithelium[54], and these cells subsequently migrate to gastric lymph nodes, where they suppress the development of T_H cells into T_H1 cells. DC-SIGN-mediated uptake of *H. pylori* is a rapid process, leaving non-DC-SIGN-binding bacteria (red) behind in the mucus layer. Even when, after a certain time, all DC-SIGN-binding *H. pylori* would have been removed from the gastric mucosa, new DC-SIGN-binding variants, which continually arise during bacterial replication, might maintain a certain level of suppression of development into T_H1 cells. *H. pylori*-specific T_H1 and T_H2 cells home to the gastric mucosa, where they establish T_H1- and T_H2-cell micro-niches. In asymptomatic chronic gastritis, T_H1-cell microenvironments might coexist with T_H1- and T_H2-cell microenvironments (see also the T-cell clones depicted in FIG. 1). In T_H1-cell microenvironments, the *H. pylori* population might be partially killed by T cells, through IL-12- and possibly IFN-γ-dependent mechanisms[29,86,87]. However, the T_H1-cell response also increases gastritis[87] and might free nutritious compounds for *H. pylori*. In T_H1- and T_H2-cell micro-niches, gastric damage is less severe, and *H. pylori* might thrive and persist in the absence of a strong T_H1-cell response.

of Lewis antigens by *H. pylori*, leading to suppression of the T_H1-cell response, can be regarded as a bacterial trait that is mutually beneficial for both host and pathogen, facilitating the switch from a T_H1-cell response to a mixed T_H1- and T_H2-cell response. The observation that most *H. pylori* strains show phase-variable expression of Lewis antigens[43,73] further supports the hypothesis that the host could selectively allow survival of *H. pylori* strains with DC-SIGN-binding ability — in micro-niches, for example[4]. The result of these host–pathogen interactions is a local balance between T_H1- and T_H2-cell responses that 'fits' the host and favours persistent colonization of *H. pylori* in the presence of mild and non-atrophic gastritis (FIG. 4b).

Within the host population, however, there might be a subgroup that pays a price, in the form of gastric autoimmunity, for persistent *H. pylori* infection. Healthy mice have subclinical numbers of H^+,K^+-ATPase-specific T cells, which have escaped negative selection as a result of the absence of H^+,K^+-ATPase in the thymus[71]. However, immune tolerance is maintained by $CD4^+CD25^+$ regulatory T cells in these animals. On infection with *H. pylori*, the H^+,K^+-ATPase-specific T cells can become activated and then clonally expand, owing to the chronic inflammatory environment and the increased antigen-presentation capability of epithelial cells (including presentation of the abundant autoantigen H^+,K^+-ATPase). Direct evidence that *H. pylori* infection can initiate gastric autoimmunity when subclinical numbers of H^+,K^+-ATPase-specific T cells are present was recently obtained using a mouse model (P. A. Gleeson, personal communication). The onset of AIG in these animals does not necessarily involve molecular mimicry between H^+,K^+-ATPase and *H. pylori* antigens but, instead, might depend on the chronic inflammation in the stomach that is induced by *H. pylori* and the loss of tolerance to gastric H^+,K^+-ATPase[74] (FIG. 5a). Also, in healthy, uninfected humans, H^+,K^+-ATPase-specific T cells can be present, as reflected by the occasional presence of H^+,K^+-ATPase-specific antibodies[63]. *H. pylori*-induced chronic gastritis alone might be sufficient to partially breakdown gastric mucosal tolerance, as shown by the presence of H^+,K^+-ATPase-specific antibodies in ~30% of people infected with *H. pylori*. This indicates that *H. pylori*-associated inflammation can initiate gastric autoimmunity and full-blown AIG only in those individuals in whom tolerance is sufficiently hampered.

In addition, in a subgroup of *H. pylori*-infected individuals, AIG could arise by molecular mimicry involving HLA-DR molecules with peptide-binding sites suitable for the presentation of *H. pylori*-derived peptides to, and the activation of, H^+,K^+-ATPase-specific crossreactive T cells[68] (FIG. 5b).

Open questions and future directions

Phase variation drives diversification of *H. pylori in vivo*, resulting in a mixture of bacteria that either bind DC-SIGN or escape from binding DC-SIGN, depending on the carbohydrate structures that are expressed[39]. As such, phase variation could have an important role in persistence of *H. pylori*. Experimental infection of rhesus monkeys showed that, in the initial stage of colonization, individual hosts are susceptible to distinct *H. pylori* strains[26]. Does this imply that human hosts can encounter *H. pylori* without being colonized, unless the ingested strain is suitably adapted to its potential host? Another intriguing question is whether the ratio of DC-SIGN-binding to non-DC-SIGN-binding bacteria within the bacterial population has any direct influence on the degree of downmodulation of the T_H1-cell response and therefore on persistence. In other words, is T_H1-cell-response suppression by *H. pylori* twice as strong when the bacterial population contains 20% DC-SIGN-binding variants as when the bacterial pool contains only 10% DC-SIGN-binding variants? A tempting explanation for the role of *H. pylori* phase variation is that, in hosts that are genetically prone to a strong T_H1-cell response, selection occurs in favour of a population of *H. pylori* in which most bacteria express carbohydrates that bind DC-SIGN and suppress the T_H1-cell response. Alternatively, there might not be a direct correlation between the proportion of an *H. pylori* population that can bind DC-SIGN and the level of T_H1-cell-response suppression. DC-SIGN-binding variants of *H. pylori* are taken up more rapidly by DCs than variants that do not bind DC-SIGN (REF. 39, and M.B. and A. Engering, unpublished observations). In that respect, a small subpopulation of bacteria that targets DC-SIGN might be sufficient to modulate the immune response, because DCs start to migrate away from the mucosa, towards lymph nodes, as soon as they have sampled DC-SIGN-binding bacteria. Few DCs are detectable in gastric sections from *H. pylori*-infected or -uninfected individuals[39]. We propose that DC-SIGN-binding variants that arise continually during *H. pylori* proliferation, even when small in number, could be sufficient to maintain immune modulation through the carbohydrate structures of their LPS.

In our opinion, future studies of asymptomatic persistence of *H. pylori* will be the most informative if they combine analysis of carbohydrate-expression profiles and DC-SIGN-binding capacity of *H. pylori* with an understanding of genetic polymorphisms in immune-response genes of the host (BOX 1).

Pathogens deliver several signals to DCs. It has been shown that C-type lectins such as DC-SIGN and the mannose receptor

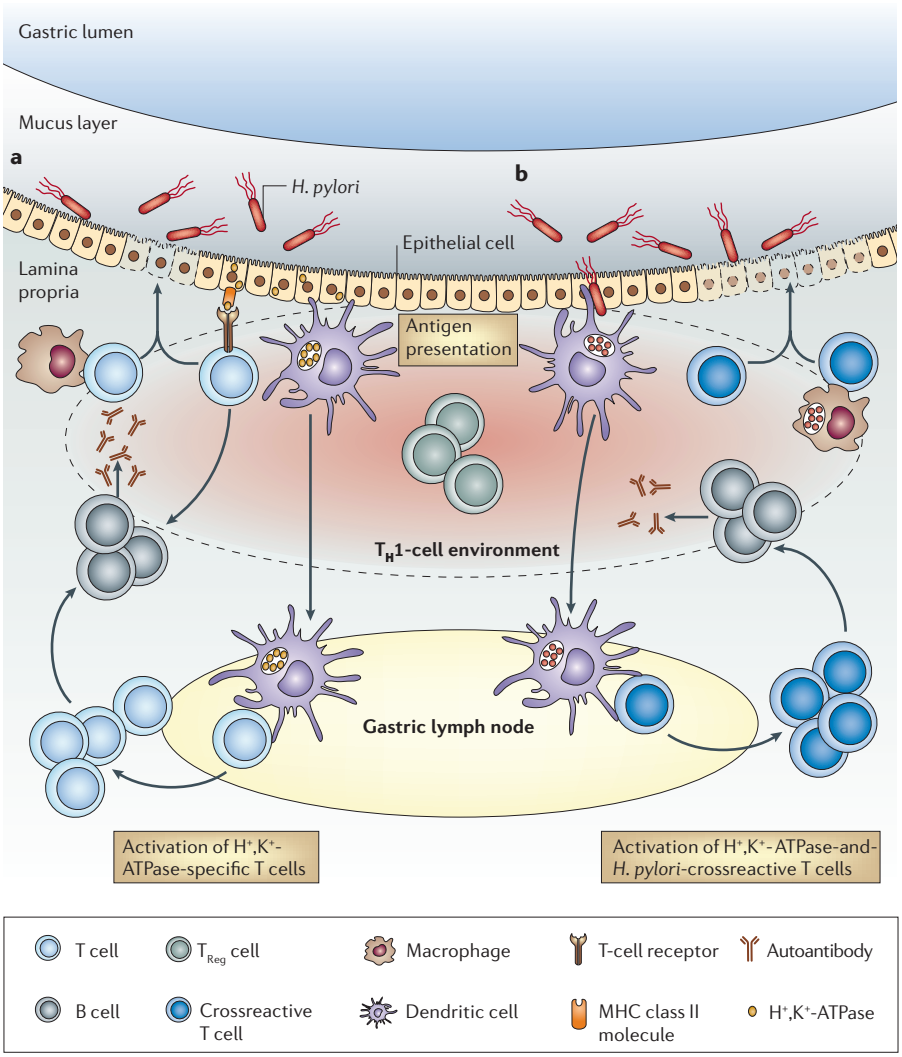

Figure 5 | **Chronic gastric inflammation induced by *Helicobacter pylori* could lead to local autoimmune responses. a** | Of patients with asymptomatic chronic gastritis, 20–30% develop autoantibodies specific for gastric H^+,K^+-ATPase. In T helper 1 (T_H1)-cell environments (FIG. 4), gastric epithelial cells might gain antigen-presenting functions in the presence of *Helicobacter pylori*. Tolerance to H^+,K^+-ATPase, which is probably maintained by $CD4^+CD25^+$ regulatory T (T_{Reg}) cells, might be reduced, and small numbers of H^+,K^+-ATPase-specific autoreactive T cells might then induce subclinical atrophy of the gastric corpus, reflected by the presence of H^+,K^+-ATPase-specific antibodies. In some individuals, tolerance to H^+,K^+-ATPase might disappear completely, leading to full-blown gastric autoimmunity and eradication of *H. pylori* as a result of the loss of its ecological niche. Trafficking of H^+,K^+-ATPase-loaded dendritic cells between the gastric epithelium and the gastric lymph nodes could be important for the maintenance of tolerance to H^+,K^+-ATPase during health, but this trafficking is strongly increased in mice that develop autoimmune gastritis[88]. **b** | Genetically susceptible individuals might develop gastric autoimmunity through molecular mimicry between the α-subunit of H^+,K^+-ATPase and *H. pylori* proteins (FIG. 3). When the human leukocyte antigen DR (HLA-DR) alleles of an *H. pylori*-infected individual facilitate presentation of *H. pylori*-derived peptides that are structurally similar to H^+,K^+-ATPase, crossreactive T cells could become activated, leading to destruction of parietal cells, which contain and present H^+,K^+-ATPase.

Box 1 | **Important research questions**

- Persistence of *Helicobacter pylori* seems to require a controlled and balanced inflammation. Certain levels of epithelial-cell death, gastric inflammation and vasodilation are required to release nutrients that are necessary for bacterial growth, indicating that expression of neutrophil-activating protein by *H. pylori* might benefit the bacteria. Excessive inflammation, which is mediated by T helper 1 (T_H1)-cell responses, results in loss of the bacterial niche (through atrophy), but a polarized T_H2-cell response might also lead to eradication of the bacteria[10]. The molecular basis of the processes that underlie asymptomatic host–pathogen coexistence remains to be elucidated.
- The interleukins IL-4, IL-10 and IL-12 seem to have distinct roles during *H. pylori* colonization, and possibly during persistence, that depend on the genetic background of the host[25]. Unravelling the complex network of interactions between these immune-response genes, in the context of the genetic background of the host, will provide crucial information for development of an effective vaccine.
- Antigen-specific $CD4^+CD25^+$ regulatory T cells have a pivotal role in protection against autoimmune damage[82], as well as in reduction of *H. pylori* -induced inflammation and pathology in mouse models[83]. In humans, however, the role of these cells in *H. pylori* infection remains to be clarified.
- The *in vivo* relevance of the observation that *H. pylori* phase variants that bind DC-SIGN suppress T_H1-cell responses *in vitro* remains to be determined.

can collaborate with each other and with TLRs[53,75–78]. In our studies, a proportion of the *H. pylori* variants that could bind DC-SIGN through Lewis antigens could also interact with the mannose receptor[39]. *H. pylori* has also been shown to interact with TLR2 and TLR4 (REF. 79, and A. Engering, personal communication), and LPS of *H. pylori* is a likely stimulus for DC-SIGN-independent maturation of DCs[39]. Current data indicate that DC-SIGN signalling interferes with TLR-mediated activation of DCs[39,53]. In addition, TLR-triggered differentiation of monocytes into DCs influences the expression of DC-SIGN[80]. The interactions between C-type-lectin signalling and TLR-mediated responses, and how these interactions might shape the immune response, are only beginning to be understood[81]. Therefore, a panel of *H. pylori* phase variants that are genetically identical, except in carbohydrate structure, will be an important tool for analysing the collaborative signalling that occurs between DC-SIGN, the mannose receptor and TLRs in DCs.

Despite intensive studies, the host–pathogen interactions that underpin asymptomatic persistence of *H. pylori* in most infected individuals are still largely a mystery. Recent evidence indicates that expression of bacterial carbohydrate structures that bind DC-SIGN is a valuable tool that is used by several pathogens to modulate the host immune response in favour of persistence. Whether other pathogens (in addition to *H. pylori*) that target DC-SIGN to facilitate persistence can initiate or accelerate autoimmunity in genetically susceptible individuals is a crucial question that warrants further investigation.

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Competing interests statement

The authors declare no competing financial interests.

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