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Review

Relevance of fucosylation and Lewis antigen expression in the bacterial gastroduodenal pathogen *Helicobacter pylori*

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Abstract—*Helicobacter pylori* is a prevalent bacterial, gastroduodenal pathogen of humans that can express Lewis (Le) and related antigens in the O-chains of its surface lipopolysaccharide. The O-chains of *H. pylori* are commonly composed of internal Le^x units with terminal Le^x or Le^y units or, in some strains, with additional units of Le^a, Le^b, Le^c, sialyl-Le^x and H-1 antigens, as well as blood groups A and B, thereby producing a mosaicism of antigenic units expressed. The genetic determination of the Le antigen biosynthetic pathways in *H. pylori* has been studied, and despite striking functional similarity, low sequence homology occurs between the bacterial and mammalian α(1,3/4)- and α(1,2)-fucosyltransferases. Factors affecting Le antigen expression in *H. pylori*, that can influence the biological impact of this molecular mimicry, include regulation of fucosyltransferase genes through slipped-strand mispairing, the activity and expression levels of the functional enzymes, the preferences of the expressed enzyme for distinctive acceptor molecules and the availability of activated sugar intermediates. Le mimicry was initially implicated in immune evasion and gastric adaptation by the bacterium, but more recent studies show a role in gastric colonization and bacterial adhesion with galectin-3 identified as the gastric receptor for polymeric Le^x on the bacterium. From the host defence aspect, innate immune recognition of *H. pylori* by surfactant protein D is influenced by the extent of LPS fucosylation. Furthermore, Le antigen expression affects both the inflammatory response and T-cell polarization that develops after infection. Although controversial, evidence suggests that long-term *H. pylori* infection can induce autoreactive anti-Le antibodies cross-reacting with the gastric mucosa, in part leading to the development of gastric atrophy. Thus, Le antigen expression and fucosylation in *H. pylori* have multiple biological effects on pathogenesis and disease outcome.

Keywords: *Helicobacter pylori*; Lewis antigens; Fucosylation; Fucosyltransferases; Molecular mimicry; Bacterial pathogenesis

Contents

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1. Introduction

Helicobacter pylori is a Gram-negative bacterium that colonizes human gastric mucosa, and is one of the most common bacterial pathogens worldwide, with a prevalence of up to 90% in developing countries.[1] Infection once established can persist for life if left untreated, and although only 30% of those infected are clinically symptomatic, infection is associated with active inflammation in the gastric mucosa.[1,2] Infection outcome is diverse and includes the development and recurrence of gastritis, gastric and duodenal ulcers, and an increased risk of gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma.[3] The cell envelope of *H. pylori*, like those of other Gram-negative bacteria, contains lipopolysaccharides (LPSs) which are important pathogenic and virulence factors of *H. pylori* with properties contributing to the severity and chronicity of this infection.[4] In general, LPSs are a family of phosphorylated lipoglycans found in the outer membrane of Gram-negative bacteria, generally possessing potent immunomodulating and immunostimulating properties.[5,6] Although components of the outer membrane, these molecules can be released by multiplying or disintegrating bacteria, as well as by the blebbing of outer membrane vesicles from the cell surface of *H. pylori*.[7]

As surface antigens (O-antigens) of *H. pylori*, these molecules are capable of inducing an antibody response, structural variation can be used as the basis for serotyping strains.[8,9] In addition, this structural variation can be detected using non-immunoglobin factors such as lectins to allow strain differentiation in a lectin typing scheme.[10,11] Like members of the *Enterobacteriaceae*, exemplified by *Escherichia coli*, *H. pylori* produces high-molecular mass (smooth form) LPS composed of an outermost saccharide moiety, divided into the O-chain and core oligosaccharide (OS) regions, covalently linked to a lipid moiety, termed lipid A, that anchors the molecule in the outer leaflet of the outer membrane.[6,12–14] Each of these domains has differing structural and biological properties.[6,12,14,15]

Extensive studies on the bioactivities of *H. pylori* LPS have revealed significantly lower endotoxic and immunological activities when compared with enterobacterial LPS as the gold standard.[4,16,17] Consistent with the hypothesis that the structure of lipid A endows *H. pylori* LPS with low immuno-activities,[18] detailed structural studies have found underphosphorylation, underacylation and substitution by long chain fatty acids in this lipid A,[13,19,20] which based on established structure–bioactivity relationships of lipid A molecules[5,12] are likely to translate into reduced immunological activities. As to the core OS, in conjunction with a 25 kDa protein adhesin,[21] that has been confirmed to be produced in vivo,[22] *H. pylori* LPS can bind laminin which is an important extracellular matrix glycoprotein found in the basement membrane.[23] Moreover, the LPS-laminin binding may inhibit recognition of the glycoprotein by the laminin receptor (67 kDa integrin) on gastric epithelial cells.[24] This interaction and also that of *H. pylori* with other extracellular matrix proteins[25] can play an important role in the loss of gastric mucosal integrity,[26] and along with other soluble factors of *H. pylori* contribute to development of gastric leakiness.[27] In addition, *H. pylori* isolates, particularly those isolated from duodenal ulcer patients, stimulate pepsinogen secretion,[28,29] a precursor of the enzyme pepsin, which is considered an aggressive mucolytic factor in the development of duodenal ulcer disease. Of note, the serological response against the core OS of LPS is more developed in duodenal ulcer patients than gastritis patients, reflecting the availability of core structures for the above interactions.[30] The identity of the structures within the core OS of LPS required for these interactions has been determined.[31–33]

The expression of Lewis (Le) and related blood group antigens in the O-chains of *H. pylori* LPS[16,17,33,34] has led to intensive investigations of the biosynthesis of these human antigens by *H. pylori* and the implications of their expression for pathogenesis and virulence of the bacterium. The aim of the present report is to review the structural and biological relevance of fucosylation and expression of these antigens by *H. pylori*.

2. Le antigens in humans and *H. pylori*

2.1. Le antigens in humans

Le antigens are biochemically related to the ABH blood groups of humans that are formed by the sequential

addition of fucose, galactose and *N*-acetylgalactosamine to the backbone carbohydrate chains of both lipids and proteins. These antigens are constituted from two types of backbone structures, containing galactose (Gal) and *N*-acetylglucosamine (GlcNAc), so-called type 1 chains [composed of Gal-β(1,3)-GlcNAc] giving rise to Lewis^a (Le^a), sialyl-Le^a and Lewis^b (Le^b), and type 2 chains [composed of Gal-β(1,4)-GlcNAc, also termed *N*-acetyllactosamine (LacNAc)] giving rise to Lewis^x (Le^x), sialyl-Le^x and Lewis^y (Le^y) (Fig. 1). In addition to the genes controlling the synthesis of these structures, another gene locus controls the expression of the antigens in secretions and on various cell types.[35] The terms secretor and non-secretor indicate the capacity of an individual to secrete such substances; secretors produce ABH, Le^b and Le^y, whereas non-secretors produce Le^a and Le^x in their saliva.[36]

In the human stomach, Le^a and Le^b are mainly expressed on the surface and foveolar epithelia, whereas Le^x and Le^y are predominantly expressed by the mucous, chief and parietal cells of the gastric glands,[37] though the physical separation of Le antigens along the gastric pits is not always obvious.[38] More specifically, in non-secretors the surface and foveolar epithelia express Le^a, whereas these epithelia express Le^b and some Le^y in secretors.[37,39] Irrespective of secretor status of the individual, the glandular epithelium lacks type 1 antigens (Le^a and Le^b) and expresses type 2 antigens (Le^x and Le^y), though in non-secretors Le^y expression is weaker in the glands.[39] Thus, a variety of Le antigens are expressed within the ecological niches of *H. pylori*.

2.2. Le antigens in *H. pylori* LPS

Generally, the O-chains of *H. pylori* clinical isolates have a poly-LacNAc chain decorated with multiple lateral α-L-fucose residues forming internal Le^x determinants with terminal Le^x or Le^y determinants (Fig. 2) or, in some strains with additional, glucose or galactose residues.[32,40–49] Furthermore, Le^a, Le^b, sialyl-Le^x and H-1 determinants have been structurally described in other strains,[48–50] as well as the related blood groups A and B,[34,53] but occur in association with Le^x and LacNAc chains. However, repeated subculturing of *H. pylori* isolates on conventional solid media can induce the production of rough-form LPS lacking an O-chain,[19] and also occurs in certain culture collection strains that have been much passaged in vitro.[13] Although strains bearing rough-form LPS can revert to smooth-LPS production when grown in liquid media,[19,54] the molecular and environmental factors influencing smooth/rough variation of this LPS have not been determined.

Expression of Le^x or Le^y determinants is a common property of (80–90%) *H. pylori* strains from various geographical regions worldwide that have been screened using anti-Le antibodies as probes.[9,55–57] Nevertheless, serological analysis can under-estimate Le^x and Le^y expression in a population of strains as some strains that are non-typeable with anti-Le antibodies have been shown to express these antigens when examined in structural studies.[46] Importantly, false-negative results are dependent upon the serological assay format used when testing strains.[58] Although it had been suggested that Asian isolates express predominantly type 1 (Le^a, Le^b, H-1) antigens compared to Western strains (predominantly expressing type 2 Le^x or Le^y determinants),[52] the former have been detected in European isolates along with Le^x and Le^y expression.[57] Hence, a mosaicism of Le antigen and blood group expression can occur in the same O-chain, and thereby, along with some variability in the core OS, gives rise to antigenic diversity that can be detected by antibody and lectin probing.[9–11]

On the other hand, there are non-typeable strains that show no reaction with anti-Le^x, Le^y, Le^a and Le^b

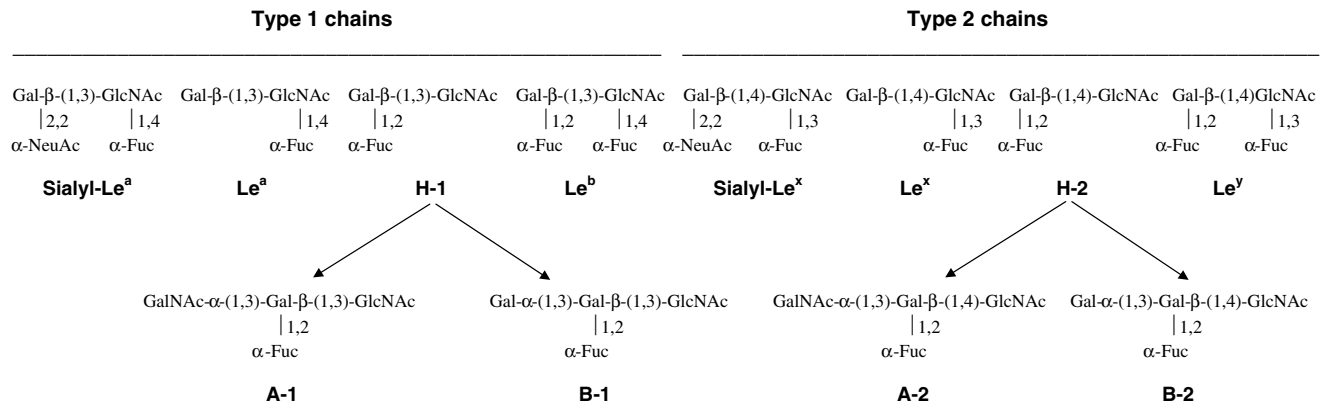

Figure 1. Structural relationship between Le and A, B and H blood group antigens. Abbreviations: Fuc, fucose; Gal, galactose; GalNAc, *N*-acetylgalactosamine; Glc, glucose; GlcNAc, *N*-acetylglucosamine; NeuAc, *N*-acetylneuraminic (sialic acid). Except for fucose, which is in the L-configuration, all sugars possess the D-configuration.

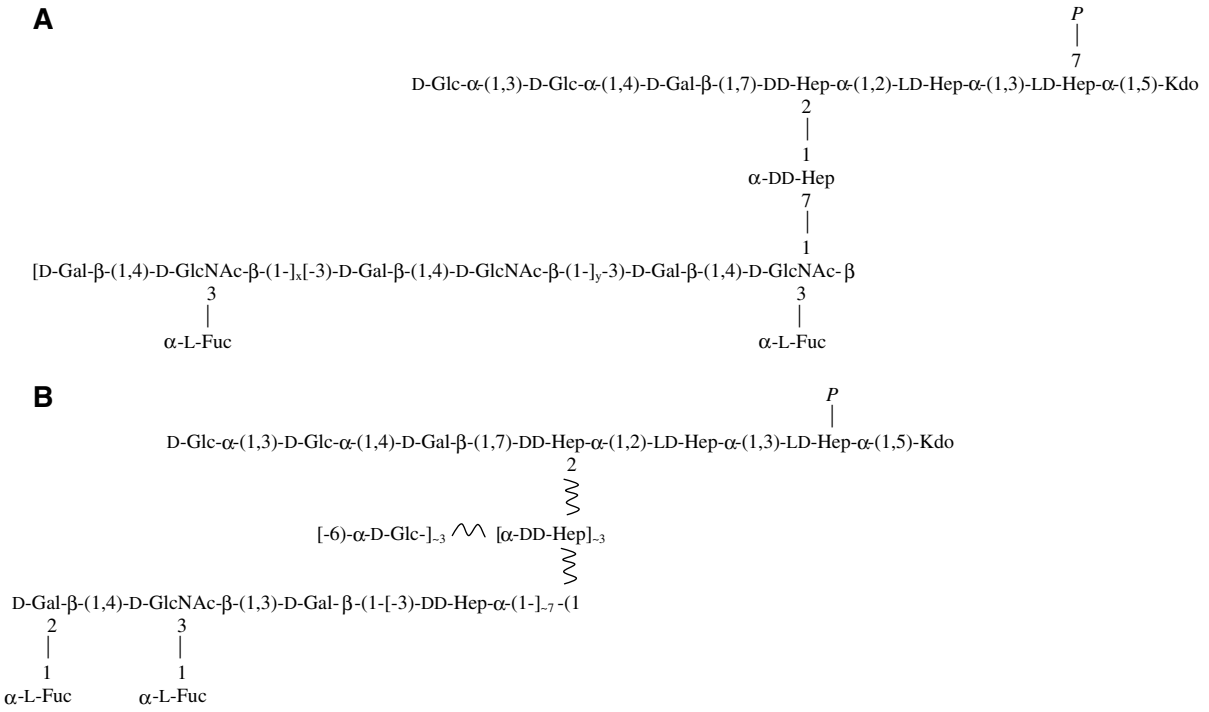

Figure 2. Proposed structure of the saccharide components of LPSs of *H. pylori* strains (A) NCTC 11637[40,42] and (B) MO19.[43] Abbreviations: Fuc, fucose; Gal, galactose; Glc, glucose; GlcNAc, *N*-acetylglucosamine; DD-Hep, D-*glycero*-D-*manno*-heptose; LD-Hep, L-*glycero*-D-*manno*-heptose; Kdo, 3-deoxy-D-*manno*-oct-2-ulosonic acid. Except for LD-Hep, and fucose which is in the L-configuration, all other sugars possess the D-configuration. In some strains of *H. pylori*, the core is substituted by phosphorylethanolamine rather than a phosphate (*P*) group.[46–49,51]

antibodies[9] emphasizing that not all strains express Le antigens.[17,33] This is particularly true for those strains associated with asymptomatic infection.[59–61] Detailed structural analysis has shown that some of these strains possess O-chains composed of L- and D-rhamnose and 3-*C*-methyl-D-mannose (Fig. 3A), a sugar not previously found in Nature.[59] Other strains have been shown to produce heptoglycans of 2- and 3-linked α-DD-heptose units[60,61] (Fig. 3B) or a glucan chain of alternating 2- and 3-monosubstituted α-D-glucose residues[62] (Fig. 3C). The absence of Le antigen mimicry in asymptomatic strains,[59–61] and animal studies in which a genetically modified *H. pylori* strain lacking Le antigen expression failed to induce gastritis compared to the parental strain,[63] support the view that the Le antigen-expressing O-chain contributes directly to disease development.

2.3. Biosynthesis of Le antigen-mimicking O-chains

Comparative examination of the two genome sequences of *H. pylori* 26695 and J99[64,65] have revealed important and useful information on LPS biosynthesis, and the conservancy of many LPS biosynthesis genes. The latter has also been confirmed with the more recent availability of the genome sequence of a chronic atrophic gastritis isolate of *H. pylori*.[66] Throughout this review, the standardized gene designations of strains 26695 (HP) and J99 (JHP) will be used.

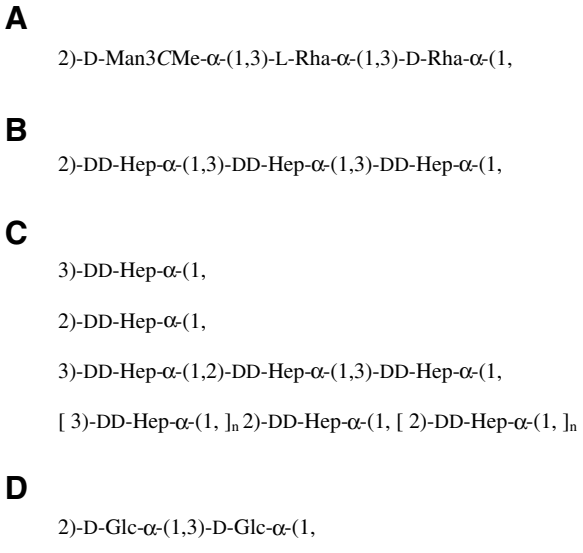

Figure 3. Proposed structure of the repeat units of O-chains of LPSs of *H. pylori* strains (A) D1, D3 and D6,[59] and (B) D2, D4 and D5,[60] (C) Hp1C2, Hp12C2, Hp62C, Hp7A, Hp75A, Hp77C and HpPJ1,[61] and (D) serotype O2.[62] Abbreviations: DD-Hep, D-*glycero*-D-*manno*-heptose; Man3*C*Me, 3-*C*-methyl-mannose; Rha, rhamnose.

For the synthesis of the LacNAc backbone of type 2 antigens such as Le^x and Le^y (Fig. 1), the β(1,4)-galactosyltransferase gene (HP0826/JHP0765) of *H. pylori* has been identified, cloned and shown to encode an enzyme that efficiently produces LacNAc units.[67,68] Moreover, as expected, knockouts in this gene produce a truncated LPS lacking an O-chain.[68] Since *H. pylori* lacks galactokinase and cannot utilize exogenous galactose, UDP-galactose-4-epimerase (encoded by *galE*, HP0360/JHP1020) is a prerequisite for the biosynthesis of UDP-galactose that is required in O-chain synthesis, and thus, *H. pylori* knockout mutants in *galE* result in the truncation of LPS.[16,69–71] Also, the gene encoding the β(1,3)-glucosaminyltransferase (HP1105/JHP1032) that is required for type 2 chain synthesis has been identified.[72] Although of low homology, an orthologue of *neuA* (HP0326/JHP309) encoding CMP-*N*-acetylneuraminic acid synthetase was suggested to be involved in sialyl-Le^x expression by *H. pylori*[73] and, supporting this, sialyl-Le^x has been confirmed to occur in the O-chain of a strain of *H. pylori*.[51]

Unlike many enterobacterial species (e.g., *E. coli* and *Salmonella enterica sv. typhimurium*), the genes for LPS synthesis are not clustered but are scattered throughout the *H. pylori* genome, with the exception of those (*rfbM*, *rfbD* and *wbcJ* orthologues) encoding proteins involved in GDP-fucose synthesis.[64,65,74] The latter acts as the donor of fucose in the fucosyltransferase reactions required for Le antigen synthesis, and is itself synthesized in two main steps by a dehydratase and then an epimerase–reductase. Genomic analysis of strains 26695 and J99 has identified the orthologues of *rfbM* (HP0043/JHP0037) predicted to encode a GDP-D-mannose pyrophosphorylase, *rfbD* (HP0044/JHP0038) a GDP-D-mannose dehydratase, and *wbcJ* (HP0045/JHP0039) an epimerase–reductase responsible for the final conversion of GDP-mannose to GDP-fucose.[73,74] Insertional mutations in *rfbM* and *wbcJ* have confirmed their role in fucosylation of the O-chains.[69,75] Nonetheless, due to the potential biological importance of fucosylation and Le antigen mimicry in the *H. pylori* O-chains, attention has focused on the fucosyltransferases (FucTs) of *H. pylori*.

3. FucTs

FucTs belong to the glycosyltransferase superfamily, in the category of Carbohydrate-Active enZYmes (CAZY) (http://www.CAZY.org/fam/acc_GT.html), and catalyse the reactions in which a fucose residue is transferred from the donor-activated sugar (GDP-fucose) to the acceptor molecule. Based on the site of Fuc addition, FucTs are classified as α(1,2)-, α(1,3/4)-, α(1,6)- and O-FucTs, and are expressed in prokaryotes and eukaryotes.[76]

3.1. Mammalian and *H. pylori* FucTs

Mammalian α(1,2)- and α(1,3/4)- FucTs are involved in the last steps of synthesis of ABH and Le-related structures, and likewise, *H. pylori* strains have α(1,2)- and α(1,3/4)-FucTs that are involved in Le antigen synthesis in LPS.[33,77–81] The α(1,2)-FucTs transfer Fuc to the terminal Gal residue of type 1 [Gal-β(1,3)-GlcNAc] and type 2 [Gal-β(1,4)-GlcNAc] chains in an α(1,2)-linkage, and α(1,3/4)-FucTs add Fuc to the subterminal GlcNAc moiety of type 2 or 1 chains in α(1,3)- and α(1,4)-linkages, respectively (Fig. 1). As in humans, the biosynthesis of Le^x in *H. pylori* is strictly ordered. Experiments using *H. pylori* extracts and acceptor molecules for fucosylation, with subsequent analyses of the enzyme reaction products, have shown that the addition of β(1,4)-linked Gal is followed by that of α(1,3)-linked Fuc.[82] It is important to note that *H. pylori* O-chains can contain incompletely fucosylated LacNAc units and evidence suggests that the *H. pylori* O-chain is synthesized by the sequential addition of monosaccharides[40–43,73] which has been described in only a few bacterial species, e.g., *Campylobacter fetus*,[83,84] rather than by the polymerization of repeating units to form the O-chain as used in the majority of Gram-negative bacteria.[12] In the case of difucosylated Le antigens (i.e., Le^b and Le^y), synthesis can occur via two pathways: (i) terminal fucosylation involving α(1,2)-linkage and then subterminal fucosylation by α(1,3)- or α(1,4)-linkage; or (ii) subterminal followed by terminal fucosylation. Mammalian cells predominantly use the former pathway[85] whereas the latter mechanism of synthesis is primarily used by *H. pylori*.[86,87]

3.2. *H. pylori* α(1,3/4)-FucTs

The *H. pylori* genome contains two paralogous genes, termed *futA* and *futB* (HP0379/JHP1002 and HP0651/JHP0596).[64,65,74] Such genes encode enzymes with either α(1,3)- and/or α(1,4)-FucT activities, which thereby use type 2 and/or type 1 acceptor molecules, respectively.[77,78,81,86] The α(1,3/4)-FucTs of *H. pylori* NCTC 11637 and NCTC 11639 (*futB* and *futA*, respectively) have exclusive α(1,3)-activity[77,78] like human FucT IV. Those of *H. pylori* UA948 (*futA*) and UA1111 (*futA* and *futB*) possess both α(1,3)- and/or α(1,4)-FucT activities,[81,86] and like human FucT V, *H. pylori* UA948 FutA has preference for the type 2 acceptor molecule.[81] *H. pylori* DSM6709 has been reported to contain primarily α(1,4)-FucT activity and only limited α(1,3)-activity,[88] like human FucT III. Thus, the activity and specificity of these enzymes may vary between the two paralogues in one strain, as well as between the orthologues in different strains.

DNA motifs near the 5′-end of these genes at two distinct polynucleotide repeats (polyA-polyC) have been

deduced to indicate regulation through slipped-strand repair.[33,65] These polynucleotide tracts have a higher frequency of addition or deletion of one or more base pairs during DNA replication than the normal mutation frequency, leading to an on/off status of the gene.[87] Hence, the slipped-strand mispairing in intragenic poly-C tract regions results in alternate reading frames and an on/off switch influencing FucT expression that gives rise to *H. pylori* subclones with different Le glycosylation patterns.[89–91] Experimentally, mutation in the polyA or polyC tract leading to the on/off status of the gene has been demonstrated in *futA* of *H. pylori* J99 and NCTC 11639[87] and in *futB* of *H. pylori* NCTC 11637.[89] The occurrence of these Le-expressing subclones, has been demonstrated not only in laboratory strains,[89] but also in clinical isolates from different regions of the human stomach and over the course of the infection.[90,91] However, the length of polynucleotide tracts does not always correlate with Le^x or Le^y expression in *H. pylori* clinical isolates,[92] indicating that post-translational events and the availability of activated sugar intermediates, in addition to an active enzyme, are important for the expression of the Le phenotype.

A comparison of α(1,3/4)-FucTs of different *H. pylori* strains demonstrates that these α(1,3/4)-FucTs show >70% sequence identity, with a highly conserved internal catalytic domain, but divergent N- and C-terminii.[81] Alignment of the *H. pylori* α(1,3/4)-FucTs with the corresponding mammalian enzyme has demonstrated significant homology only in a very short region within the catalytic domain where the two α(1,3/4)-FucT motifs are localized.[77,78] Despite this, these two enzyme families appear to share functional similarities, with a conserved mechanistic and structural basis for fucose transfer.[76] Kinetic studies have shown that *H. pylori* α(1,3/4)-FucTs are comparable to their mammalian counterparts.[88,93] Similar to human α(1,3/4)-FucTs, the hydroxyl group of carbon-6 of Gal in type 1 and 2 acceptor molecules is also essential for the equivalent *H. pylori* enzymatic activity.[93] *H. pylori* α(1,3/4)-FucTs are able to use both the sialylated type 1 and 2 acceptors, like the human enzymes.[88,93] Also, like human α(1,3/4)-FucTs, those of *H. pylori* catalyse fucose transfer following a sequential mechanism, whereby binding of the donor molecule occurs first followed by that of the acceptor.[93]

H. pylori α(1,3/4)-FucTs lack the N-terminal cytosolic tail and transmembrane domain of the mammalian counterpart enzymes that are type II membrane proteins,[94] but instead have a heptad-repeat region at the C-terminus that is absent from mammalian enzymes.[77,78] This heptad-repeat region contains a leucine-zipper motif potentially mediating dimer formation,[77,78] and the ability of *H. pylori* α(1,3/4)-FucTs to form dimers has been confirmed experimentally.[93,95] In addition, thermal denaturation studies have confirmed that the heptad-repeats facilitate protein folding, and hence help to maintain a stable protein structure that is aligned with the dimer formation.[95] The C-terminal heptad-repeat region consists of a variable number of DD/NLRV/INY tandem repeats. Nilsson et al.[91] found that the heptad-repeat number of FutA and FutB in *H. pylori* isolates correlated with the size of the O-chain being fucosylated. The data supports a molecular ruler mechanism for how *H. pylori* varies its fucosylation pattern within the O-chain, where one heptad-repeat in the enzyme corresponds to one LacNAc unit in the O-chain. Thus, a model has been proposed in which FutA and FutB form heterodimers or homodimers in which the number of heptad-repeats controls the active fucosylation site from a fixed point and thus determines the size of the O-chain that is fucosylated.[91] Moreover, the number of heptad-repeats in FutA and FutB appears to affect subsequent α(1,2)-fucosylation by FutC, probably reflecting the availability of terminal Le^x substrates on the O-chains.

The amphipathic helices of the *H. pylori* α(1,3/4)-FucTs have been deduced to act as membrane anchors with the hydrophobic face embedded in the membrane and positive charges interacting with phospholipid headgroups.[76,96] Using domain swapping and site-directed mutagenesis, the C-terminal hypervariable region of *H. pylori* α(1,3/4)-FucTs, which is immediately upstream of the heptad-repeat region, and particularly an aromatic residue (Tyr), was shown to be responsible for type 1 acceptor recognition.[93] Overall, mammalian and *H. pylori* FucTs share similar domain architecture but with opposite topology as reviewed elsewhere.[76] Hence, the membrane anchor region (N-terminus of mammalian but C-terminus of *H. pylori* FucTs) can be truncated without significant loss of enzymatic activity, but the removal of a small number of amino acid residues from the opposite terminus (C-terminus in mammalian and N-terminus in *H. pylori*) of FucTs can almost completely abolish enzymatic activity.[77,93,95]

3.3. *H. pylori* α(1,2)-FucTs

Preliminary studies found no putative gene for α(1,2)-FucTs in the genome sequence of *H. pylori* 26695.[64,73,97] A truncated gene (HP0094) with a C14 tract was found and in silico insertion of a C-G pair yielded a full-length protein with strong homology to other α(1,2)-FucTs.[73] Further analysis of the genome of strain 26695 showed that it contained two truncated reading frames (HP0093 and HP0094), compared to that of *H. pylori* J99 (JHP0086), and the gene was assigned as *futC*.[64,65,73,74] The α(1,2)-FucTs from *H. pylori* strains share very high sequence similarity (about 95%) with one another, but very low similarity (up to 22%) with mammalian enzymes.[98] *H. pylori* α(1,2)-FucTs lack the N-terminal cytosolic tail and the transmembrane domain compared to mammalian enzymes, and in one

H. pylori strain α(1,2)-FucT has been demonstrated to be located in the cytoplasm as a soluble protein.[79]

Similar to the *futA* and *futB* genes, *futC* also contains the polyA-polyC tract near the 5′-end, and during DNA replication, the addition or deletion of one or more base pairs within these tracts occurs, thereby leading to an on/off status of the gene.[79,80,87,89,99,100] Along with these short repeat sequences, imperfect TAA- or GAA- or AAA-repeats occur at the mid-region of *futC*, immediately downstream of the poly-C tract.[80] In addition to these signature sequences, an internal Shine-Dalgrano-like context, a heptamer (AAAAAAG) and the downstream potential stem-loop structure are present also.[76,80] The hypermutable sequence provides a possibility of frequent shifting into and out of coding frame by a polymerase slippage mechanism. During the translation process, ribosome slippage can occur to the −1 reading frame within the heptamer at a frequency of 50%, thereby encoding a full-length α(1,2)-FucT rather than two truncated products. Such a mechanism has been confirmed to occur in *H. pylori* 26695.[80] Moreover, *futC* genes in some *H. pylori* strains do not possess a valid promoter region which results in the inability to express the α(1,2)-FucT.[76,80] In certain strains, insertions in the hypermutable region generate a frameshift mutation which cannot be compensated for by a translation frameshift cassette.[80] Thus, the on/off status of the *H. pylori futC* can be controlled at both the translational and transcriptional levels. Overall, the α(1,2)-FucT activity has been found to be much lower generally than that of the α(1,3/4)-FucTs in the same *H. pylori* strain.[79,80,87] Furthermore, as discussed above, the number of heptad-repeats in FutA and FutB appears to affect the subsequent α(1,2)-fucosylation by FutC.[91]

The frequency of phase variation resulting in on/off switching of Le antigen expression by *H. pylori* is about 0.2–0.5%.[89,99,100] To predict the Le glycosylation pattern in a *H. pylori* O-chain it is necessary to consider the on/off status of the *futA*, *futB* and *futC* genes, the number of heptad-repeats, the activity and expression levels of the functional enzymes, the preferences of the expressed enzyme for distinctive acceptor molecules, and the availability of activated sugar intermediates.

4. Roles of LPS-expressed Le antigens in *H. pylori* pathogenesis

Biologically, *H. pylori* Le expression has been implicated in evading the immune response upon initial infection and in influencing bacterial colonization and adhesion,[16,17,33] and with the progress of chronic infection it has been suggested to contribute to gastric autoimmunity that leads to gastric atrophy, a precursor state of gastric cancer.[7,101–103] Although the latter has been considered controversial,[16,17,104] more recent evidence supports a contributing role of Le mimicry-induced auto-antibodies, as well as peptide-directed autoreactive T-cells,[105] in the development of gastric atrophy.

4.1. Gastric adaptation

Based on the known expression of Le antigens in the gastric mucosa, it was proposed that bacterial molecular mimicry and its adaptation could provide an escape for *H. pylori* from the host humoral response by preventing the formation of antibodies shared by the host and bacterium.[106,107] As detailed previously,[16,106–109] some human population and primate model studies supported this concept, whereas others have not.

Specifically, a study examining the relationship between infecting *H. pylori* isolates and gastric Lewis expression in the human host, as determined by erythrocyte Le(a,b) phenotype (i.e., secretor status), reported that the relative expression of Le^x or Le^y by *H. pylori* strains corresponded to Le(a+,b−) and Le(a−,b+) blood group phenotypes, respectively, of the hosts from whom the individual strains were isolated.[106] Therefore, it was suggested that because Le^a and Le^b are isoforms of Le^x and Le^y, respectively, and surface and foveolar epithelia express Le^a in non-secretors and Le^b in secretors that the correlations seen were a form of adaptation or selection of *H. pylori* strains to the gastric mucosa of the individual host. In an animal model, using experimental infection of rhesus monkeys, a *H. pylori* strain isolated from infected monkeys that are of the secretor phenotype expressed more Le^y than Le^x, whereas conversely the same infective strain expressed more Le^x than Le^y when isolated following the colonization of non-secretor monkeys.[108,109] Thus, in this experimental system, Le^x/Le^y expression by *H. pylori* depended on the host. However, unlike in humans,[101,102] no data is available on whether the infected monkeys form antibodies against Le^x and Le^y that would act as a selective pressure.

On the other hand, studies in another patient population, performed in an identical manner to that above, did not find a correlation between Le^x/Le^y on *H. pylori* isolates and host Lewis expression.[57] Also, a further study which directly examined Lewis expression in the gastric mucosa of infected patients using immunohistochemical techniques did not find a correlation between bacterial Le^x/Le^y expression and that on gastric epithelial cells of the human hosts.[110] The discrepancies between the patient studies may reflect the human study populations.[16,103] Of considerable importance is that 26% of the human study population, where the correlation was observed, was of the recessive Lewis phenotype, Le(a−,b−), based on erythrocyte testing.[106] This is higher than that usually observed for a Caucasian or European population, from which the two other study populations were drawn. Using salivary testing

rather than erythrocyte typing would have allowed the distribution of this phenotypically recessive group of patients to their true secretor/non-secretor phenotype and would have influenced the correlative results. In the two other studies,[57,110] no patients of a recessive phenotype were observed. Finally, despite high anti-LPS antibody titres, *H. pylori* eradication and protection is mediated by cellular not humoral immunity,[110] and this challenges whether anti-LPS antibodies in humans can act as a selective pressure.

Importantly, it has been demonstrated that *H. pylori* strains expressing Le^x and strains expressing Le^y can be isolated from the same host,[90] and extensive diversity of Le^x and Le^y in O-chains can occur over time and in different regions of the human stomach,[91] thereby apparently contradicting the hypothesis of bacterial Le antigen adaptation to that of gastric Le expression-related secretor status of the host. Notwithstanding this, the diversity of Le expression by *H. pylori* subclonal isolates in one host may reflect an ability and potential of the bacterium to adapt to differing microniches and environmental conditions within the human stomach.[47,112] For example, pH may vary between the differing regions of the stomach (i.e., the antrum versus the corpus) as well as in the microbial niches of the mucosa (i.e., on the luminal surface of gastric mucus, within the mucus and also on the epithelial cell surface) and pH has been shown to influence the relative Le^x/Le^y expression by *H. pylori*.[47]

4.2. Role in adhesion and colonization

Loss of O-chain and Le^x expression have been reported of importance for in vivo colonization by *H. pylori* in mice.[16,68,70,107] Construction of an isogenic *H. pylori galE* (HP0360/JHP1020) mutant, affecting galactose incorporation, resulted in truncation of LPS and loss of O-chain expression, which led to a loss of ability of this strain to colonize a number of mouse strains compared with the Le^x-expressing parental strain.[70] Similarly, mutation of a β(1,4)-galactosyltransferase gene (HP0826/JHP0765) affected the synthesis of the O-chain backbone and resulted in less efficient colonization of the murine stomach by the engineered strain.[68] Inactivation of the *rfbM* gene (HP0043/JHP0037), encoding a GDP-D-mannose pyrophosphorylase required for GDP-fucose synthesis, resulted in a fucose-lacking O-chain (thus, an i-antigen-expressing chain), which reduced mouse colonization[70] and ablated interaction with the human gastric mucosa of biopsy specimens in situ.[69] Although one study reported no significant changes in mouse colonization by *H. pylori* mutants with ablated Le^x/Le^y-expression,[117] this study used C3H/HeJ ($TLR4^{-/-}$) mice, the so-called LPS non-responder mice, which may not be the most appropriate animal model of infection since *H. pylori* LPS induces TLR signalling.[4] In contrast, a mutated *H. pylori* strain with a double knockout in both α(1,3)-FucTs did not colonize a more appropriate mouse model compared to the Le^x/Le^y-expressing parental strain in another study.[107] Collectively, these data indicate that not only O-chain occurrence is required, but specifically Le^x expression is also required for colonization.

Polymeric Le^x expression in *H. pylori* O-chains has been shown to mediate, at least in part, *H. pylori* adhesion to the human antral gastric mucosa[69] and whose gastric receptor has been identified as the galactoside-binding lectin, galectin-3.[113] Emphasizing the role of Le^x in adhesion–recognition phenomena, fluorescently labelled latex beads bearing polymeric Le^x demonstrated the same tropic binding to human gastric tissue as fluorescently labelled *H. pylori* bacteria.[69] Nevertheless, *H. pylori* adhesion is mediated by a number of adhesins[19,114,115] with polymeric Le^x considered to play a distinct role.[116]

Consistent with an adhesion role, strains with a high expression of Le^x cause a higher colonization density in patients than those with weaker expression.[57] By assisting bacterial adhesion and interaction with the gastric mucosa, like other adhesins, bacterial Le^x expression may enhance the delivery of secreted products into the gastric mucosa thereby promoting chemotaxis and leucocyte infiltration.[16,118] Noteworthy are the differences in pH within mucus and on the gastric cell surface (pH 2 on the luminal side of the gastric mucus layer to almost pH 7 on the cell surface), and that environmental pH influences Le^x expression by *H. pylori*.[47] Optimal expression of Le^x occurs at neutral pH, as on the cell surface, but reduced expression occurs at lower pH, thus modulating the expression of the Le^x-adhesin and allowing free-swimming *H. pylori* in the mucus layer. The latter acts as a reservoir for continued bacterial infection and the observed on/off switching of fucosyltransferase activities, and hence phase variation in Le expression,[89] would be in accord with this mechanism of continued infection.

4.3. Influence on the innate and inflammatory response

Counterbalancing the gastric adhesion role of the O-chains, surfactant protein D (SP-D), which is a C-type lectin involved in antibody-independent pathogen recognition and clearance,[119] binds *H. pylori* LPS resulting in bacterial immobilization and aggregation.[49,120] Levels of expression of SP-D are significantly increased in *H. pylori*-associated antral gastritis compared to normal gastric mucosa, and SP-D expression co-localizes with *H. pylori* organisms.[120] Also, experiments in $SP\text{-}D^{-/-}$ mice revealed that *Helicobacter* colonization was more common in the absence of SP-D,[119] underscoring an important influence of SP-D binding on the establishment of infection. To evade this important mechanism

of innate immune recognition of *H. pylori*, escape variants can arise within the bacterial cell population with modifications in O-chain glycosylation decreasing their interaction with SP-D.[49]

Likewise, strain-to-strain variability in Le expression was shown to modulate the interaction of *H. pylori* LPS with the cellular innate immune receptor DC-SIGN (another C-type lectin) on gastric dendritic cells which contributes to changed T-cell polarization after innate immune activation.[121] In the effector phase of an immune response, different T-cell subsets, called T-helper-1- (Th1-) and T-helper-2- (Th2-) cells, expand; Th1-cells promote proinflammatory cell-mediated immunity, whereas Th2-cells promote humoral immunity that induces B-cells to produce antibodies.[111] Dendritic cells, in response to *H. pylori*, secrete a range of cytokines; preferentially interleukin- (IL-) 12 that induces a Th1-response, but also lesser amounts of IL-6 and IL-10.[122] Since Lewis antigen expression is subject to phase variation,[89] a significant proportion of Lewis-negative variants can occur within an isolated population of *H. pylori.* Le-negative variants of *H. pylori* escape binding to dendritic cells and induce a strong Th1-cell response.[121] In contrast, *H. pylori* variants that express Le^x/Le^y can bind to DC-SIGN on dendritic cells and enhance the production of IL-10 which promotes a Th2-cell response and blocking of Th1-cell activation. Thus, a polarized Th1-effect can change to a mixed Th1-Th2-cell response through the extent of Le antigen-DC-SIGN interaction.[121] This modulation of the host response allows a switch from an acute infection response to the one that allows the development of chronic infection since the humoral response and antibody production are not associated with *H. pylori* eradication or protection. A similar alteration in the T-cell response, based on the over-expression of IL-10 and induced by polymeric Le^x, has been observed in infection by eggs of the parasitic worm *Schistosoma mansoni*.[123]

Furthermore, it has been suggested that expression of both Le^x and Le^y by *H. pylori* could aid the persistence of proinflammatory *cagA*-positive strains, i.e., strains carrying the *cag* pathogenicity island-encoding type IV secretion system, in patients with more aggressive inflammation and pathologies. This was based on the observations in a sample human population that *H. pylori* isolates positive for Le^x/Le^y were predominantly *cagA*-positive and that a genetically engineered *cagA*-negative strain had diminished expression of Le^y.[55] On the other hand, other studies using a different human population showed a lack of association between *cagA* status and Le^x/Le^y expression.[56,57] These discrepancies in findings were suggested to be due to the adaptation of *H. pylori* strains with differing attributes to different human populations,[16] a conclusion which was later supported in a pan-European study examining *cagA* status and Le expression of isolates.[103] Thus, depending on the human host population, Le^x/Le^y expression by *H. pylori* may aid the persistence of more aggressive strains.

4.4. Putative role in the development of gastric autoimmunity

Although it has become apparent that bacterial colonization density and the ensuing inflammatory response can be influenced by host expression of ABO and Le^a blood group determinants,[124] bacterial Le^x expression is associated with peptic ulcer disease,[56] and is statistically related to neutrophil infiltration.[57] Interestingly, experiments indicate that neutrophils are a potential target recognized by anti-Le^x antibodies[101] which can be generated during prolonged *H. pylori* infection.[7,101,102] Neutrophils express CD15 (Le^x) on the members of the adhesion-promoting glycoprotein family (CD11/CD18) and it has been speculated that cross-linking of CD15 by *H. pylori*-induced anti-Le^x auto-antibodies could potentiate polymorph adhesiveness to the endothelium.[101,123] Supporting this, anti-Le^x monoclonal antibodies, including those induced by *H. pylori*, can activate and cause enhanced adherence of these cells, which may result in tissue damage and inflammation.[101,123,125] Nevertheless, this remains an open question for further clinical investigation.

Furthermore, during chronic infection by *H. pylori*, expression of Le^y has been implicated in the pathogenesis of atrophic gastritis, a precursor pathological state before the development of gastric cancer, through the induction of autoreactive antibodies against the gastric mucosa.[16,33,101,125–128] Moreover, such auto-antibodies have been found in patients with atrophic gastritis and gastric cancer.[7,102,127] In particular, the β-chain of the gastric proton pump (H^+,K^+-ATPase) contained within parietal cell canaliculi is glycosylated predominantly by Le^y and has been implicated in the pathogenic autoimmune responses in atrophic gastritis.[16,101,123] Anti-Le^y *H. pylori*-induced antibodies were shown to react with the purified human and murine β-chain.[101] In an adoptive transfer experiment, growth in mice of a *H. pylori*-induced anti-Le^y-secreting hybridoma resulted in gastric histopathological changes consistent with those of gastritis.[101,126] Also, anti-Le and anti-parietal cell-related antibodies were induced in a transgenic mouse model of *H. pylori* infection in which gastric pathology developed.[126] Collectively, these data had been taken to indicate that Le^y mimicry plays a role in the induction of *H. pylori*-related atrophic gastritis,[16,17,33,123] but since these experiments were performed largely in mice, questions were raised as to whether a similar phenomenon occurred in humans.[104]

However, a correlation has been established between the presence of auto-antibodies in *H. pylori*-infected

individuals and with the degree of gastric infiltration, numbers of inflammatory cells, and with glandular atrophy.[127,128] In the case of patients suffering from severe gastric atrophy, *H. pylori* isolates were found more likely to express Le^x/Le^y antigens, whereas isolates from individuals with near-normal mucosa were less likely to express these antigens and to induce auto-antibodies in experimental animals.[127] Additionally, the anti-canalicular auto-antibodies have been shown to increase significantly in mice patients with the duration of *H. pylori* gastritis and to correlate with gastric corpus atrophy.[129,130] Nevertheless, it has been suggested that these pathogenic antibodies are the consequence, rather than the causative factor in atrophic gastritis, based on the classical model of organ-specific autoimmunity in which there is a central role for increased autoantigen (i.e., Le) presentation in the gastric mucosa because of *H. pylori*-induced damage.[104,131]

This view has been largely based on certain absorption experiments attempting to abolish the reactivity of auto-antibodies from human sera using *H. pylori* preparations. In one such series of experiments using *H. pylori* lysates a decrease in anti-gastric autoreactivity was observed,[126] whereas in another no significant reduction was observed,[131] and anti-H^+,K^+-ATPase serum auto-antibodies were not absorbed with *H. pylori* whole cells in another study.[132] Experimentally, however, many of these studies did not use matched isolates with the sera of the patient from whom they were isolated. Secondly, patient sera were shown to react with recombinant H^+,K^+-ATPase expressed in *Xenopus* oocytes which was deduced to indicate that the reactivity observed was based on protein epitopes rather than Le glycosylation,[133] although the nature of glycosylation occurring in the oocytes was not confirmed.[16,33]

Of particular note, it has been established that antigen presentation and the serological assay format that is used play an important role in the successful detection of Le antigen expression displayed in the O-chains of *H. pylori*.[58,101,102,134] Conversely, the use of synthetic glycoconjugates in solution or in solid phase does not optimally detect *H. pylori*-induced anti-Le antibodies,[102] which has led to the inability to detect such antibodies in certain patient studies.[135] For these reasons, it has been speculated that the form of antigenic presentation of Le^y could potentially influence the ability to absorb *H. pylori*-derived anti-Le antibodies from patient sera.[16,33] Of note, the outer membrane of *H. pylori* undergoes blebbing, producing vesicles, the membranes of which contain LPS with associated Le^x/Le^y expression.[7] In absorption experiments, using these *H. pylori*-derived vesicles and sera from gastric cancer patients, it has been possible to remove (83–100%) anti-Le^y antibodies and cause significant reduction, though not complete ablation, of antibodies reactive with the canaliculi of parietal cells.[7] Thus, other protein-based epitopes of the H^+,K^+-ATPase must also be involved in the auto-reactivity observed.[131]

Based upon the available knowledge, it can be deduced that the mechanisms underlying anti-gastric auto-antibody production likely include molecular mimicry between *H. pylori*-expressed Le^y and the gastric mucosa, as well as facilitated exposition and presentation of the bacterial epitopes to recruited immune cells within the gastric mucosa. This, in turn, will be influenced by host immune-regulation and environmental factors. A cross-reactive antibody such as anti-Le^y may initiate damage to the proton pump, with subsequent alteration in acid output.[101] Changes in acid output may regulate LPS glycosylation, thereby influencing the LPS structure of the infecting *H. pylori* strain such that phase variation occurs in the expression of Le epitopes.[47] Hence, a secondary reduction in autoreactive antibody production could occur, thereby confounding the interpretation of the role of *H. pylori*-induced anti-Le antibodies. Moreover, the initiation of the inflammatory cascade against *H. pylori* and the activities of the virulence factors produced by the bacterium,[19,114,115] and even anti-Le^x antibodies in complement-mediated lysis of host cells,[101,123] would cause histological damage in the gastric mucosa. Subsequently, host structural epitopes would become exposed and presented to the immune system to further drive the autoreactive response, through humoral and T-cell responses to peptides.[104,105,131] Therefore, it is unlikely that the presence of anti-Le^x/Le^y antibodies would be solely responsible for both the initiation and maintenance of the anti-gastric autoimmune response in *H. pylori*-positive patients.

5. Concluding remarks and future directions

H. pylori has a well-established ability to evade and even subvert innate and adaptive immune responses during long-term infection[2,4] and certain properties of *H. pylori* LPS, including those associated with the O-chain, contribute to these. Similar to *H. pylori*, many colonizers of the gut, including commensals and pathogens, have developed mechanisms to vary LPS structure and thus may subvert recognition by innate immune receptors.[136] This underlines the importance of LPS as a surface structure and immune recognition ligand. Some insights have already been gained into the influence of acidic pH, as occurs in the human stomach, on fucosylation of the O-chain,[47,80] but how these relate to or occur during in vivo colonization needs testing. Importantly, modulation of LPS structure could influence immune recognition within the gastric mucosa, and thus, influence colonization and its disease outcome. The interaction of *H. pylori* LPS with SP-D and the influence of phase variation in FucTs on *H. pylori*-SP-D interaction serves

as an obvious paradigm.[49] Likewise is the influence of Le antigen expression and fucosylation on T-cell polarization and the inflammatory response that develops after infection.[111,121] On the other hand, although controversial, evidence suggests that long-term *H. pylori* infection can induce autoreactive anti-Le antibodies cross-reacting with the gastric proton pump of parietal (acid-secreting) cells, thus contributing to their destruction, and the development of gastric atrophy which is a precursor pathological state before gastric cancer development.[7,101,102,126,127] Thus, Le antigen expression and fucosylation in *H. pylori* should be viewed as producing multiple biological effects in pathogenesis and disease outcome, rather than affecting a single response or attribute.

Nonetheless, despite extensive investigations on the molecular mechanisms of regulation of the FucTs that are required for Le antigen synthesis, the environmental factors regulating the expression of these enzymes, and their functioning, require more detailed examination. With the availability of the crystal structures of *H. pylori* FucTs[137] further insights should be gained into the molecular recognition and functioning of these enzymes.

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