Commentaries

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H. pylori and Lewis antigens

Lipopolysaccharide (LPS) of many *Helicobacter pylori* strains expresses Lewis antigens (Le^x, Le^y, Le^a, Le^b) which are similar to those expressed by gastric epithelial cells ("molecular mimicry").¹ In addition, *H. pylori* LPS displays phase variation in these antigens—that is, the high frequency, reversible switching of phenotype²; for instance, a strain that expresses Le^x may yield variants that express Le^y. As yet, no definite role has been assigned to these Lewis antigens, nor to phase variation, in the pathogenesis of gastric disease.

In this issue of *Gut* (see page 18), Zheng and colleagues³ report that *H. pylori* strains isolated from Asian peptic ulcer patients express two or more Lewis antigens more often than strains from non-ulcer dyspepsia patients (89.6 v 73.2%; p=0.035). What could be the link between *H. pylori* Lewis antigen expression and the development of pathology in the host?

- (1) Lewis antigens induce pathogenic antibodies. On infection, *H. pylori* LPS may induce anti-Le^{x/y} antibodies that bind not only to bacteria but also to host gastric epithelium, followed by complement fixation and tissue destruction.¹ Indeed, *H. pylori* infection of mice induces autoreactive anti-Le^{x/y} antibodies.¹ However, although high titres of antibodies to *H. pylori* LPS are found in the serum of infected patients,¹ these antibodies are not autoreactive and not directed against Le^{x/y} (see Yokota and colleagues⁴).
- (2) Lewis antigen mimicry provides persistence through immune evasion. Analogous to ABO blood group antigens, a host that expresses Le^x would be able to form anti-Le^y antibodies but not antibodies directed at Le^x. Consequently, Le^x positive bacteria infecting a Le^x positive host would escape immune attack and be able to persist while an Le^y positive strain would not be able to persist. Experimental infection in monkeys confirms this concept: a *H. pylori* strain isolated from Le^y positive monkeys expressed mainly Le^y; the same strain expressed mainly Le^x after colonisation of Le^x positive animals.⁵ This indicates that expression of the *H. pylori* Lewis phenotype depends on the host; adaptation can occur by means of phase variation² followed by selection through anti-Le^{x/y} antibodies. Two of three studies in humans, however, failed to demonstrate the existence of a correlation between the phenotypes of the host and pathogen.⁶ Moreover, it has been shown that strains expressing Le^x, and strains expressing Le^y, can be isolated from the same patient.⁷ Finally, a shift in *H. pylori* Le^{x/y} antigen expression would be driven by anti-Le^{x/y} antibodies and there is no evidence that these are formed in infected patients.⁴
- (3) Lewis antigens are involved in adhesion and colonisation. Expression of Le^{x/y} is crucial for in vivo colonisation of mice: mutants with inactivated β 1,4-galactosyltransferase⁸ or α 3-fucosyltransferase genes (S L Martin, submitted) expressed no Le^{x/y} and colonised less well than their Le^{x/y} positive parent strains.

How would Le^{x/y} expression affect colonisation? Recent data suggest that Le^{x/y} plays a role in adhesion. A monoclonal antibody (Mab) specific for *H. pylori* LPS inhibited adhesion to gastric epithelial cells⁹; this Mab was specific for Le^x (B J Appelmek, H Yamaguchi, unpublished). Strains knocked out in *galE* or *rfaM*, genes involved in the biosynthesis of Le^x, did not adhere to gastric tissue sections.¹⁰ In addition, synthetic Le^x bound to human gastric epithelial cells from some hosts¹⁰ but not from all (T Boren, unpublished). Studies in gastritis patients demonstrated that *H. pylori* strains that strongly expressed Le^{x/y} caused a higher colonisation density than strains that expressed Le^{x/y} weakly.⁶ These data suggest that expression of Le^x enhances colonisation through increasing adherence. They also predict the existence of a gastric Le^x binding lectin. Experimental studies confirmed this: Le^x binding polypeptides of 16–29 kDa are found in gastric epithelial cells¹¹; the identity of these proteins is unknown. Independent studies have shown that surfactant protein D, a 120 kDa lectin belonging to the innate defence system and expressed in the stomach, can also interact with *H. pylori* LPS¹²; which part of the LPS is recognised is unknown.

Thus a role for LPS/Le^{x/y} in adherence seems likely. This role is not absolute: Le^{x/y} negative mutants can adhere as strongly as their Le^{x/y} positive parents (T Boren, unpublished), and Le^{x/y} negative strains colonise human hosts well.¹³ Thus an Le^{x/y}-lectin interaction may contribute to adhesion for only some strains and only in part of the hosts. For example, the adhesin BabA¹⁴ is important for adhesion of *H. pylori* and recognises Le^b expressed by gastric epithelium; only *H. pylori* strains that do not express BabA or strains that colonise humans that do not express Le^b might need their Le^{x/y} antigens for adhesion. In this concept, LPS phase variation allows detachment of bacteria not expressing Le^{x/y} and hence transmission to another host; subsequently, switch back variants expressing Le^{x/y} adhere and colonise a new host.

Adhesion of *H. pylori* has clinical relevance: strains from ulcer patients more often express BabA compared with strains from gastritis patients.¹⁴ What is the link between adherence and development of host pathology? Firstly, increased adherence may lead to an increased bacterial burden. Secondly, studies in mice showed that increased adherence did not necessarily lead to increased colonisation density but to closer contact between bacteria and gastric epithelial cells.¹⁵ A more intimate contact enhances the cross talk between microorganism and host and may lead to activation of transcription factor NF- κ B and host signal transduction pathways. This induces IL-8 production and inflammation, and finally, ulceration. This sequence of events is in agreement with data that show that increased Le^x expression in *H. pylori* is associated with increased neutrophil infiltration.⁶

In summary, current data, including those provided by Zheng and colleagues,³ are in agreement with the hypothesis that *H. pylori* LPS Lewis antigens play a role in adhesion and inflammation; LPS phase variation may be essential for host-to-host transmission. To conclude, after several years of intensive research on *H. pylori* LPS structure, genetics, and biosynthesis we may finally start to understand the biological role of *H. pylori* Lewis antigens.

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A little rest and relaxation

In healthy subjects and in patients with mild to moderate gastro-oesophageal reflux disease, gastro-oesophageal reflux occurs mainly during transient lower oesophageal sphincter (LOS) relaxations.^{1–3} Transient LOS relaxations are a neural reflex, organised in the brain stem, with efferent and afferent pathways travelling in the vagus nerve.³ Distention of the proximal stomach is a major trigger for the reflex to occur, although stimulation of the pharynx or the larynx may also contribute.³ It is clear that the initiation of the reflex requires activation of gastric mechanoreceptors.

Because of their pivotal role in the occurrence of gastro-oesophageal reflux, the neurophysiology and pharmacology of transient LOS relaxations are topics of intense ongoing research. Atropine is one of the drugs that were recently shown to inhibit gastro-oesophageal reflux by inhibiting transient LOS relaxations.⁴ It is unclear if atropine is acting at the level of the stomach, by altering the mechanosensitivity of the proximal stomach, or at the level of the brain stem, by interfering with central integrative processing.

Figure 1 Schematic representation of types of gastric mechanoreceptors (schematically drawn as coils and positioned in reference to gastric smooth muscle cells) and their response to distention, muscular relaxation, and muscular contraction.

In this issue of *Gut*, Lidums and colleagues⁵ used a gastric barostat procedure to study the influence of atropine on proximal gastric tone and on the occurrence of transient LOS relaxations in healthy subjects (see page 30). Atropine caused prolonged relaxation of the proximal stomach after a meal and decreased the rate of transient LOS relaxations. By comparing the effects on proximal gastric tone and on the rate of transient LOS relaxations, the authors concluded that the inhibitory effect of atropine on transient LOS relaxations was most likely at the central level.

The nature of the fundic mechanical receptors involved in triggering postprandial transient LOS relaxations is still poorly understood. Based on animal studies it has been proposed that mechanoreceptors are positioned either in series or in parallel to smooth muscle fibres. In parallel, mechanoreceptors respond to stimuli that elongate the stomach wall while in series, mechanoreceptors respond to stimuli that increase the tension within the stomach wall.^{6,7} Figure 1 illustrates the responses of tension mechanoreceptors (in series) and elongation mechanoreceptors (in parallel) to different stimuli (distention, relaxation, and contraction of smooth muscle). In series, mechanoreceptors are activated during distension and during contraction against a resistance; they are inactivated during relaxation. In parallel, mechanoreceptors are activated during distension and relaxation and are inactivated during contraction. Animal studies suggest that gastric mechanoreceptors with afferents in the vagal pathways are primarily tension receptors.⁷

Gastric distention, the best studied trigger of transient LOS relaxations in humans, results in elongation as well as increased wall tension of the stomach. Hence it is unclear which of these two types of mechanoreceptors initiates the afferent limb of the reflex. Administration of atropine prolongs relaxation and thus elongation of the proximal stomach after a meal. Hence if the mechanoreceptors involved in triggering transient LOS relaxations were elongation receptors, atropine should have enhanced transient LOS relaxations.

The influence of administration of atropine on activation of tension receptors in the gastric wall after a meal is less clear. Attempts have been made to differentiate tension from elongation following the law of Laplace:

$$T = \Delta P (r/2)$$

where T =wall tension, P =pressure, and r =radius.⁸ In the present study, intragastric pressure was kept constant by

the barostat device, and postprandial intrabag volume, and thus the radius, was maintained higher after atropine. If calculated according to the simplified law of Laplace, a higher wall tension after a meal results from administration of atropine. However, use of the Laplace formula requires a number of assumptions that are not necessarily fulfilled under the given circumstances: the wall of the stomach is infinitely thin, the intragastric balloon and stomach are assumed to have a perfect spherical shape, and the pressure external to the stomach is known and is evenly distributed. Most importantly, wall tension in the proximal stomach has both passive and active components. The Laplace formulas, most suitable in a passive tension model, do not take into account the modulatory effects of changes in the contractile state of the proximal stomach which may occur reflexly or in response to neurohumoral or pharmacological modulation. During a process of gastric relaxation, such as that which occurs during accommodation of a meal or after administration of atropine, wall tension is likely to fall because of diminution in the active component of wall tension. In the presence of an intragastric barostat bag, keeping intragastric pressure constant and above the level of intra-abdominal pressure and thus providing a continuous drive for low level distension, it is impossible to estimate the true effect on wall tension. However, it seems reasonable to assume that, under the conditions of the present study, wall tension was not decreased after a meal in the presence of atropine. Consequently, if the mechanoreceptors involved in triggering transient LOS relaxations were tension receptors, atropine should have enhanced transient LOS relaxations in the present study.

Based on these underlying assumptions, the authors correctly state that whatever the mechanoreceptors involved, under the conditions of the present study, relaxation of the proximal stomach would be expected to increase the rate of transient LOS relaxations. In support of this notion, fundic relaxation induced by sumatriptan was associated with an increased rate of transient LOS relaxations and gastro-oesophageal reflux.^{9,10} Furthermore, slow infusion of lipids into the duodenum induces gastric relaxation and increases the rate of transient LOS relaxations, both of which are prevented by pretreatment with a cholecystokinin receptor antagonist.¹¹ Hence atropine induced inhibition of transient LOS relaxations is most likely due to its action at the level of the central nervous system. This does not preclude the possibility of influ-

encing the occurrence of transient LOS relaxations through an effect on proximal gastric tone. A successful peripherally acting approach would, however, require knowledge of the type of mechanoreceptor involved in triggering transient LOS relaxations. In the case of a tension receptor, relaxation of the proximal stomach should decrease the occurrence of transient LOS relaxations. In the case of an elongation receptor, enhanced gastric emptying or redistribution of a meal to the distal stomach should decrease the rate of transient LOS relaxations. Identification of the type of mechanoreceptor involved in triggering transient LOS relaxations in humans is likely to require an innovative approach, avoiding the undistinguishing influence of gastric distention.

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MMPs in the gut: inflammation hits the matrix

Two papers in this issue of *Gut* focus on RNA and protein levels of matrix metalloproteinases (MMPs) in diagnostic biopsies of patients with inflammatory bowel disease (see pages 57 and 63).^{1,2} Both reports are congruent in that in ulcerated lesions, MMP-3 mRNA, which encodes a key enzyme in matrix degradation, is highly increased (up to 15- and 100-fold, depending on the methodology used), paralleled by a more moderate increase in MMP-3 protein levels, whereas there is little change in levels of the physiological inhibitor of most MMPs, tissue inhibitor of metalloproteinases 1 (TIMP-1). Thus the elevated ratio of MMP-3 over TIMP-1 would favour matrix degradation. Similar data were obtained for MMP-1/TIMP-1 (degrada-

tion of fibrillar collagens), MMP-2/TIMP-2 (degradation of basement membrane collagen and denatured collagens), and for membrane-type MMP-1 (MMP-14) which can activate MMP-2.²

Whereas matrix dissolution is plausible for ulcerative colitis (UC), it comes as a surprise that comparable levels of expression for MMPs and MMP/TIMP-1 ratios were found in ulcers of Crohn's disease (CD) which rather leads to intestinal fibrosis. As expression of several collagens which are the major components of scar tissue was also not different between lesions of CD and UC,³ the factors that determine fibrogenesis (that is, enhanced matrix deposition) and fibrolysis (that is, stimulated matrix removal) in the gut remain to be determined.

What can we learn from these studies in terms of pathophysiology, and what diagnostic and therapeutic consequences can be derived? Firstly, reliable micromethods were established that allowed both RNA and protein quantification of several MMPs and TIMPs from a single

Figure 1 Metalloproteinases (MMPs) and their cellular sources in the intestine. Minor or still debated MMP expression is shown in italics. Intestinal fibroblasts appear to play the most prominent role in MMP release during mucosal inflammation and subsequent transformation. Tumour necrosis factor α (TNF- α) has been identified as a major MMP inducing cytokine. Several currently used drugs affect MMP expression and activity indirectly. MMP inhibitors may prove useful in modulating mucosal transformation.

diagnostic biopsy. Secondly, expression of various MMPs and TIMPs correlated with the histological degree of intestinal inflammation, but did not depend on its aetiology.

However, there are many explanations for the obviously quite different matrix metabolism in UC and CD. mRNA and protein quantification do not allow us to draw conclusions as to the biological activity of the MMPs, the majority of which are secreted as inactive proenzymes that have to undergo a complex proteolytic processing to become fully active. MMP processing is brought about by plasmin, furin-like proteases, or MMPs themselves (with MMP-3 and MMP-14 playing prominent roles as proactivators of other MMPs), with the proactivators again being tightly regulated.⁴ Furthermore, matrix degradation is usually restricted to small cell membrane associated compartments whereas the great mass of MMPs remains inactive and complexed to TIMPs. Thus mere quantification does not tell us anything about the temporospatial expression of the various MMPs or TIMPs.

Fortunately, the cell types responsible for MMP expression in normal and inflamed intestine *in vivo* are fairly well defined, thanks to several studies using *in situ* hybridisation, in part in combination with cell type specific markers.⁵⁻⁹ Although this knowledge may allow some extrapolation, determination of the focal proteolytic activity of individual MMPs *in vivo* defies current technology. The picture is further complicated by prominent sequestration of most MMP precursors in the matrix where many bind to certain collagens; this explains the relatively large amounts of pro-MMPs which are usually not stored intracellularly that can be extracted from tissues.^{1,2}

Despite the complexity of MMP regulation in intestinal inflammation, some *in vitro* and *in vivo* data allow us to draw more definite conclusions. Thus mice deficient in MMP-7 and MMP-11, which in the gut are almost exclusively produced by intestinal epithelial cells (but which were not measured in the two present reports), are less susceptible to genetic or chemical intestinal carcinogenesis,

and deletion of the macrophage specific metalloelastase MMP-12 prevents macrophage transmigration through basement membranes *in vivo* (reviewed by Nagase and Woessner⁴). *In vitro* stimulation of T lymphocytes in organ cultures of fetal intestine caused activation of several MMPs but only MMP-3 proved to be a key enzyme for degradation of the lamina propria extracellular matrix.¹⁰ As intestinal fibroblasts are the prime sources of this protease,^{7,8} and as tumour necrosis factor α (TNF- α), released by the T lymphocytes, is a powerful inducer of fibroblast MMP-3, this creates a plausible link between mucosal inflammation and destruction of the subepithelial matrix (fig 1).

What determines the partly divergent evolution of the lesions in UC and CD? Are there differences in the overall or localised expression of other MMPs, MMP proactivators, or of other classes of matrix degrading proteases that were not determined in the present investigations? Such differences should be expected due to the divergent cytokine profile in UC and CD which resembles a TH2 and a TH1 pattern, respectively, as cytokines are potent modulators of MMP expression and activity.

The usefulness of corticosteroids and immune suppressants such as azathioprine which act mainly on lymphocytes underscores the relevance of activated T cells, and the often dramatic improvement in complicated CD with TNF- α blockade may derive, at least in part, from inhibition of MMP activation favouring for example, closure of fistulas. TNF- α blockade does not appear to be equally effective in UC where in contrast, interferon alpha (IFN- α), a TH1-like cytokine, may be promising. The domain for quantification of MMPs, as exemplified in the two present reports, may be to monitor such therapies and, by extension of the spectrum of the molecules analysed, to find better and more specific predictors of disease activity in UC and CD. A novel therapeutic approach could be blockade of certain MMPs, such as MMP-3, by local or systemic application of synthetic MMP inhibitors. Much industrial research effort has been invested in the development of such compounds for treatment of tumours or osteoarthritic joint destruction.

Therefore, these two papers have introduced novel mucosa derived parameters which may prove useful to assess prognosis, disease activity, and treatment response in inflammatory bowel disease. By using this methodology, other genes involved in mucosal remodelling may be found that more specifically reflect the underlying aetiology. Their use in future therapeutic trials is awaited with much interest.

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Heparin and inflammation: a new use for an old GAG?

Since its discovery in 1917, heparin has been a fascinating, and in a way elusive, molecule. Heparin is a glycosaminoglycan (GAG) formed by repeated sulphated oligosaccharide units. Natural preparations of heparin (usually obtained from bovine lung or porcine intestinal mucosa) can vary in the length of the polymeric unit and therefore have different molecular weights. As such, the biological actions of this GAG can vary quantitatively between different batches of the molecule. The initial activity ascribed to heparin was its capacity to prolong the process of blood clotting, an effect due to its potentiating interaction with the natural inhibitor of thrombin, antithrombin III. These properties have led to widespread use of heparin as an anticoagulant although scant attention has been paid to other biological activities of this GAG.

This situation has changed in recent years. In fact it has long been recognised that heparin has a wide range of biological effects in addition to its well characterised anticoagulant property, including the ability to display anti-inflammatory efficacy (reviewed by Jacques¹ and Tyrrel and colleagues²). The anti-inflammatory activity of heparin has been reinforced by positive, although small, clinical trials in patients suffering from a range of inflammatory diseases, including rheumatoid arthritis³ and bronchial asthma.⁴ In addition, a number of clinical studies have recently demonstrated the anti-inflammatory activity of heparin in the treatment of inflammatory bowel disease at doses that do not produce antihemorrhagic complications (for example, see Dwarakanath and colleagues⁵). Given that it is now well recognised that different portions of the heparin molecule exhibit anti-inflammatory activity, and that a pentasaccharide sequence retains the ability to inhibit antithrombin III,⁶ it is possible that the anti-inflammatory actions of heparin are distinct from its anticoagulant activity.²

Under various experimental and clinical conditions, heparin was found to actively reduce the process of leucocyte recruitment into the site of injury or of application of

inflammatory stimuli. In this issue (see page 88), Salas *et al* provide evidence for the first in vivo mechanism responsible for the anti-migratory action of heparin.⁷ In fact, intravital microscopy techniques have allowed direct observation of inflamed microvascular beds with definition of the paradigm of white blood cell extravasation. Leucocyte interaction with the endothelium of an inflamed post-capillary venule is initially intermittent and dynamic (cell rolling), it then becomes static (firm adhesion), and is finally followed by diapedesis.⁸ Using the multipotent cytokine, tumour necrosis factor- α (TNF- α) to promote this cascade of events in vivo, Salas and colleagues⁷ reported that heparin downregulated TNF- α induced leucocyte rolling, adhesion, and migration into gut tissue without affecting changes in vascular permeability. These data extend and confirm previous studies in which heparin reduced leucocyte adhesion to vascular endothelial cells in vitro⁹ and recruitment of inflammatory cells into other tissues during an experimental inflammatory reaction.²

What is the molecular mechanism(s) responsible for the anti-migratory action of heparin? The study by Salas and colleagues⁷ begins to clarify this important issue in an in vivo context. Heparin is acidic in nature and can therefore bind many cationic proteins, including leucocyte and endothelial adhesion molecules (reviewed by Tyrrel and colleagues²). In this new study, heparin at a dose of 500 μ g/kg, which was unable to alter the partial thromboplastin time, affected TNF- α induced leucocyte extravasation without modifying TNF- α induced endothelial adhesion molecule (CD62P, CD54, and CD106) expression. This is consistent with in vitro observations that heparin inhibits adhesion of leucocytes to umbilical endothelium cells without altering expression of adhesion molecules.⁹ Treatment of rats with heparin did not modify the increase in adhesion molecules (CD11b/CD18 and CD62L) measured in polymorphonuclear leucocytes two or five hours post-TNF- α injection⁷ although increased ex vivo binding capacity of circulating neutrophils to heparin was noted. Of the three adhesion molecules tested, heparin binding occurred at least in part to the β_2 integrin CD11b/CD18, as demonstrated by displacement by a selective monoclonal antibody. These in vivo observations partially confirm the data generated in vitro, emphasising the selective interaction between heparin and CD11b/CD18, but

not with CD62L or CD11a/CD18. The authors concluded that heparin blocks CD11b mediated cellular events such as firm adhesion and in this manner this GAG can effectively interfere with the process of leucocyte extravasation that is central to the host inflammatory response.⁷

As is often the case with ground breaking studies, several other questions can be formulated as a result of this work. In particular, heparin was effective in inhibiting not only cell adhesion to and migration through the mesenteric post-capillary venule endothelium (effects easily ascribed to interference with CD11b mediated events) but it also attenuated TNF- α induced cell rolling, a phenomenon clearly independent of this β_2 integrin.⁸ Therefore, an interaction(s) other than with CD11b must be occurring between heparin and an unknown endogenous protein(s) which sustains white blood cell rolling. Future studies will address this aspect although binding to CD62L (L-selectin) can be excluded.⁷ Potential interference with CD62P (P-selectin), as demonstrated in vitro, or with selectin counterligands, may be proposed. This possibility is reinforced by the time dependency of heparin inhibition of cell rolling on the inflamed post-capillary venule endothelium (at two but not five hours post-TNF- α).

Will new anti-inflammatory drugs capable of controlling diseases such as colitis be developed out of this research on heparin? This is a pertinent question that is now being addressed in several laboratories. A number of chemically modified fractions of heparin that retain an anti-inflammatory effect have been identified, yet are lacking in anticoagulant activity.²⁻⁹ Recently, a pentasaccharide sequence containing the antithrombin binding site was described⁶ that interfered selectively with the coagulation cascade but did not produce the haemorrhagic side effects of heparin (due to the ability of the long polymer to bind platelet factor 4).²⁻⁶ In addition, pentosanpolysulphate has been introduced into clinical practice in the USA as a treatment for interstitial cystitis based on the antiadhesive effects of heparin.¹⁰ There is no reason why more successful molecules cannot be identified based on a better under-

standing of the antiadhesive action of heparin, and recent studies are addressing this aspect.⁹ The observation of Salas and colleagues⁷ will undoubtedly give impulse to this line of research.

In conclusion, the novel study published in this issue of *Gut* has shed further light on the biological activities of heparin. Fragments of this natural product may in the future lead to the development of novel drugs with a wide range of clinical uses in the treatment of inflammatory diseases, including those of the gastrointestinal system.

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