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***Helicobacter pylori*, Lewis antigens, and inflammation**

EDITOR.—In a recent issue of *Gut*, Zheng *et al* reported that peptic ulcer disease was associated with increased expression of Lewis (Le) antigens but not *cagA*, *iceA*, or *vacA* in Chinese patients infected with *Helicobacter pylori* (*Gut* 2000;47:18–22). These data raise as many questions as they answer and add further controversy to an intriguing area of microbiology and gastroenterology.

Le^x and/or Le^y blood group determinants are commonly found on the lipopolysaccharide (LPS) of *H. pylori* isolates. These determinants have been identified on approximately 80–90% of isolates from all patient series examined to date.^{1–3} In our own series, 74/84 (88%) isolates expressed either Le^x or Le^y, with 47/84 (56%) of isolates co-expressing both determinants.⁴ No difference was identified in the prevalence of Le^x or Le^y expression on *H. pylori* strains isolated from ulcer patients and those strains isolated from patients with chronic gastritis, nor was an association demonstrated between host and bacterial Lewis phenotype. Since the report of Zheng *et al* we have reviewed our raw data regarding the prevalence of strains expressing two or more Lewis antigens. We found that two or more Le determinants were present in 9/12 (75%) isolates from duodenal ulcer patients and in 4/10 (40%) isolates from gastric ulcer patients. This gives an overall prevalence of 59% (13/22 isolates) for isolates from patients with ulcer disease. Thirty four of 62 (55%) patients with histological evidence of chronic gastritis also had isolates expressing two or more Le antigens.

A significant difference between *H. pylori* strains isolated from Chinese patients and those from our population is the finding of 48/108 (44%) isolates expressing three or more Le antigens compared with 11/84 (13%) isolates from a homogenous Irish population. In our series, only one strain expressed all class 1 and class 2 Le antigens—that is, Le^x, Le^y, and their isomers Le^a and Le^b. This unusually high prevalence of three or more antigenic determinants raises the question of whether or not these differences were due to strain variation or relate to antibody specificity. Zheng *et al* also suggest that blood group A determinants have not been identified on *H. pylori* isolates. We have previously described this determinant on a *H. pylori* strain expressing both Le^x and Le^y.^{4,5} However, the role of fucosylated blood group determinants such as blood group A, B, or H type-1 on *H. pylori* LPS is as yet undetermined. This contrasts with the well established role of blood group A in *H. mustelae* mediated gastric autoimmunity in the ferret model of gastric autoimmunity.⁶

These observations on the role of expression of multiple Le determinants are interesting, given the nature of our study population where patients with ulcerative disease made up only 26% of the overall study population. These individuals were infected with strains with a relatively well conserved pattern of Le determinant expression. Both genotypic conservation and adaptation of *H. pylori* to host populations is well recognised in *H. pylori* infection. Clonality is a recently described property of this bacterium and merits discussion in relation to Le determinants. In recent studies, *H. pylori* strains isolated from 12

human hosts and 16 experimentally infected rodents showed Le expression to be highly uniform in isolates from different rodents infected for up to 20 weeks' duration.⁷ Substantial differences in Le expression were found among isolates from human patients and these differences were unrelated to the presence of random amplified polymorphic DNA sequences or *cagA* status. It has been suggested therefore that variation in *H. pylori* Le expression in genetically similar microorganisms in human subjects may provide a pool of bacterial phenotypes for the continuous selection of host adapted populations for persistence.⁷ Clonality such as that reported for Le determinants could result in the presence of antibodies against the predominant Le epitope in strains from an individual host in vivo. A previous study has suggested that in mimicking host gastric epithelium, *H. pylori* isolates not only express Le^x and Le^y, but that their relative proportion of expression corresponds to the host Le blood group phenotype (secretor status).³ We and others have found no evidence to support this hypothesis.^{4,8}

In Asian populations, where the prevalence of *cagA* genotype is high among *H. pylori* isolates, no association between *cagA* genotype and ulceration has been found.⁹ Recent data have demonstrated that the prevalence of *cagA*+ *H. pylori* isolates is 44% among a group of 1025 men from 18 British towns.¹⁰ The consequence of this prevalence to disease in the general population is uncertain. However, given the association between *cagA* and more aggressive pathology among Western populations,¹¹ the relationship between various virulence factors such as *cagA* and putative modifying or colonisation factors such as Le determinants must be clarified. Other data from our group have provided preliminary evidence that *cagA*, *vacA*, or *vacA* expression does not correlate with the level of Le antigen expression.¹² However, no large dataset is available from a homogenous Western population. The original series that suggested that *cagA* status was related to Le antigen expression had 94 isolates from 19 countries and therefore is subject to marked bias.²

Lastly, one issue that requires further clarification is the relationship between bacterial Le antigen expression and neutrophil or lymphocytic infiltrates in the host gastric mucosa. In other work, we have shown that inflammatory responses to *H. pylori* infection may be influenced at least in part by host Le^x expression.¹³ Moreover, we have demonstrated that blood group O non-secretors had a significantly higher grade of lymphocyte infiltration of the gastric mucosa compared with non-O non-secretors.⁶ We have also demonstrated that Le^x expression was associated with increased acute inflammatory infiltrates in patients with either ulcer disease (duodenal and gastric) or chronic gastritis.⁴ Based on the high prevalence of both Le^x and Le^y expression in Chinese patients, the role of inflammation needs to be explored further.

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Relation of *H. pylori* to gastric mucins and gastric surface mucous gel layer

EDITOR.—We read with considerable interest the excellent article on *Helicobacter pylori* colonisation with MUC5AC in the human stomach by Van den Brink *et al* (*Gut* 2000;46:601–7). The authors used antibodies against gastric mucin core proteins (anti-MUC6 and MUC5AC (45M1)) in conjunction with antibody against *H. pylori* and demonstrated attachment of *H. pylori* to gastric surface mucous cells and the presence of *H. pylori* in extracellular mucins derived from gastric surface mucous cells.

These findings are similar to those previously reported by us¹ using histochemical staining specific for gastric mucins (dual staining consisting of galactose-oxidase cold thionine Schiff reaction (GOTS) and paradoxical concanavalin A staining (PCS))², and immunostaining for *H. pylori*. GOTS recognises galactose or N-acetyl galactosamine residues of gastric surface mucous cell mucins and stains gastric surface mucous cell mucins blue (fig 1A). Histochemical reactivity of GOTS is identical to that of immunostaining with anti-MUC5AC (45M1) (fig 1B). PCS recognises the specific sugar residues with peripheral N-acetyl glucosamine in gastric gland mucous cells (cardiac gland cells, mucous neck cells, and pyloric gland cells) and stains gastric gland mucous cell mucins brown (fig 1A). We used tissue sections from surgically resected stomachs fixed in Carnoy's solution which has the

Figure 1 (A, B) Formalin fixed normal fundic mucosa. (A) Galactose-oxidase cold thionine Schiff reaction (GOTS) stains gastric surface mucous cell mucins blue and paradoxical concanavalin A staining (PCS) stains mucous neck cell mucins brown (dual staining of GOTS-PCS). (B) Immunostaining with anti-MUC5AC (45M1) labels gastric surface mucous cells. The reactivity of anti-MUC5AC (45M1) is identical to that of GOTS (see (A)) (immunoperoxidase method with anti-MUC5AC (45M1)). (C) Carnoy fixed gastric mucosa without *Helicobacter pylori* infection. The surface mucous gel layer had a characteristic structure consisting of laminating layers of two types of mucins; the surface mucous cell type (blue) and the gland mucous cell type (brown) (dual staining of GOTS-PCS). (D, E) Carnoy fixed gastric mucosa with *H. pylori* infection. (D) *H. pylori* is stained red by an immuno-alkaline phosphatase method with anti-*H. pylori* antibody. Numerous *H. pylori* exist in the surface mucous gel layer and on and between the surface mucous cells. In the surface mucous gel layer, *H. pylori* preferentially colonise in the layer of surface mucous cell type mucins (dual staining of GOTS-PCS followed by immunostaining for *H. pylori*). (E) *H. pylori* is stained red by an immuno-alkaline phosphatase method with anti-*H. pylori* antibody. Numerous *H. pylori* preferentially exist on and between the surface mucous cells but not on pyloric gland cells (dual staining of GOTS-PCS followed by immunostaining for *H. pylori*).

advantage of fixing the gastric surface mucous gel layer (SMGL) in paraffin embedded tissue sections.³

Dual staining of GOTS-PCS of Carnoy fixed gastric mucosa without *H. pylori* infection showed that SMGL had a characteristic structure consisting of laminating layers of two types of mucins: the surface mucous cell type and the gland mucous cell type (fig 1C).^{1,3} Dual staining of GOTS-PCS in conjunction with immunostaining for *H. pylori* in Carnoy fixed stomachs with *H. pylori* infection revealed that *H. pylori* characteristically existed on and between the surface mucous cells and in the SMGL (fig 1D).¹ In the SMGL, this organism was most often associated with the layer of surface mucous cell type mucins (fig 1D, E).¹ Receptors responsible for adherence of *H. pylori* might exist only on the plasma membrane of surface mucous cells. In the SMGL, these receptors appear to be mixed with the surface mucous cell type mucins. The SMGL inhabited by *H. pylori* revealed marked derangement of the

multilaminating structure with fragmentation of the mucin layers and formation of vacuoles (fig 1D, E).¹ After eradication of *H. pylori*, the SMGL regained the laminating structure.¹ Alteration of the SMGL found in *H. pylori* infected stomachs suggests destruction of the SMGL by bacterial lipase and protease from *H. pylori*.^{4,5}

H. pylori in the SMGL was more abundant than that attached to the surface mucous cells (fig 1D, E).¹ The SMGL appears to be the major site of *H. pylori* colonisation and may serve as a vehicle for diffusion of *H. pylori* to other sites in the stomach and duodenum.

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Reply

EDITOR,—I thank Dr Ota *et al* for their interest in our work. I was already familiar with the exquisite pictures of the laminated structure of gastric mucus by these authors. The present new data as shown, in conjunction with our previous data, shed more light on the phenomenon of *Helicobacter pylori* attachment in the stomach. Although I generally support the conclusions of Ota *et al*, the following remarks are in order.

It seems very likely from figure 1A and 1B of their letter that the surface mucous cells that produce MUC5AC (by 45M1 immunostaining) were stained blue on galactose-oxidase cold thionine Schiff reaction (GOTS) staining. This implies that the blue sublayers within the laminated extracellular mucus would also be composed of MUC5AC. Also, as the authors show in fig 1 (D, E), *H. pylori* was specifically localised to: (1) the blue layers within the mucus layer (that is, most likely MUC5AC; fig 1D); and (2) the blue stained cells (that is, most likely producing MUC5AC; fig 1D, E). Implicit in our collective data is that the brown staining layers, as demonstrated by paradoxical concanavalin A staining (PCS), are probably composed of MUC6. Collectively, these data corroborate our previous conclusion that MUC5AC and MUC5AC producing cells (and not MUC6) are the main attachment sites for *H. pylori* in the stomach. Yet final prove awaits immuno-histochemical co-localisation of MUC5AC, MUC6, and *H. pylori* in the mucus layer. As Carnoy's solution may not allow simultaneous immunochemical detection of these components, technical problems presently hamper final conclusions. In pursuing this, I think it is extremely important to concentrate on the primary gene products—that is, localisation of MUC5AC and MUC6 proteins—rather than on carbohydrates on complex

molecules. Glycosylation is a complex process that may easily become disturbed in *H. pylori* infection, for example. Thus as glycosylation may change under pathological conditions, I would urge the authors and other workers in the field to concentrate on the invariant part of the mucins: the non-O-glycosylated parts of the protein, as discussed by us previously.¹

One of the issues addressed by Ota *et al* is the correlation of *H. pylori* infection with disturbance of the laminated gastric mucus layer, as demonstrated in fig 1C versus fig 1D. In common with the authors, it is tempting to speculate that *H. pylori* has a direct role in this disturbance. Similar to most bacteria, *H. pylori* produces enzymes that in principle can degrade mucins, such as proteases and "mucinases", as referred to by the authors. I personally doubt whether *H. pylori* is directly responsible for disturbance of the mucus layer for two reasons. Firstly, as the bacterium resides in the mucus layer, what would be the benefit of destroying its own milieu? Secondly, there are data by many authors to indicate that the tissue dynamics of the gastric mucosa is altered by *H. pylori* infection. Accompanying the inflammatory response, epithelial turnover is enhanced, and there are profound shifts in cell populations within the epithelium. As a result, which was also mentioned in our previous *Gut* article, MUC6 may be over expressed in *H. pylori* infected stomach at the expense of MUC5AC expression. From our own work, and that of Byrd and colleagues,² it appears that the number of cells expressing MUC5AC declines and that concomitantly the relative number of MUC6 producing cells rises. Meanwhile, we have further data to corroborate this notion, and it appears that there is a statistically highly significant decrease in MUC5AC producing cells (Van de Bovenkamp JHB, Korteland-Van Male A, Büller HA, *et al*, unpublished data). Thus shifts in expression levels of individual mucins that constitute the mucus layer may easily explain the disturbance of the gastric mucus layer. Obviously, this type of discussion calls for a more dynamic approach to this problem. Only a detailed study of mucin biosynthesis (at the mRNA and protein levels and rate of secretion) may reveal the truth behind my speculations. At this point we will have to make do with an educated guess.

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Helicobacter pylori infection and acid secretion in patients with duodenal ulcer in Japan

EDITOR,—*Helicobacter pylori* infection affects gastric acid secretion of the host in various ways. For example, corpus gastritis and subsequent development of mucosal atrophy induced by *H. pylori* infection result in decreased acid secretion.¹ In contrast, several investigators have reported elevated acid secretion in patients with duodenal ulcer

Figure 1 Effects of *Helicobacter pylori* eradication on maximum acid output (MAO) in Japanese patients with duodenal (DU) and gastric (GU) ulcers. MAO in response to pentagastrin (6 µg) was measured before and six months after *H. pylori* eradication. * $p < 0.05$ v before eradication.

(DU) which is decreased by *H. pylori* eradication with simultaneous reduction of serum gastrin.^{2,3} Supporting these data, Iijima *et al* (*Gut* 2000;46:20-6) reported that eradication of *H. pylori* was accompanied by an increase in acid secretion in patients with gastric ulcer (GU) in whom corpus gastritis and/or atrophy are frequently observed, whereas acid secretion decreased after eradication in those with DU. However, in countries such as Japan where corpus gastritis and mucosal atrophy are common among patients infected with *H. pylori*, the situation is not as simple.

We performed a similar study by measuring basal acid output (BAO) and maximum acid output (MAO) using pentagastrin administration before and six months after eradication in Japanese patients with GU (n=21) and DU (n=22). In both patients with GU and DU, serum gastrin levels were significantly higher than those of control subjects ($p < 0.01$) before eradication; they decreased significantly after eradication (GU: 129.7 (19.2) to 94.6 (15.0) pg/ml; DU: 116 (19.8) to 90.3 (16.3) pg/ml; $p < 0.05$). These data are similar to those of Iijima *et al* (*Gut* 2000;46:20-6). In contrast with their data however we could not find any significant changes in MAO after eradication in DU patients although *H. pylori* eradication resulted in a significant increase in MAO in patients with GU (fig 1). Indeed, as shown in fig 2, changes in MAO varied from patient to patient among DU individuals. Subsequently, we attempted to elucidate the

relationship between changes in serum gastrin and MAO more precisely. As expected, in patients with GU, a positive correlation was found between the decrease in serum gastrin and increase in MAO following *H. pylori* eradication (fig 2). These data suggest that recovery of acid secretion by *H. pylori* eradication in patients with GU is responsible for the decrease in serum gastrin levels. In contrast, in patients with DU, no significant correlation was observed between changes in serum gastrin and acid secretion. When we analysed the DU data more precisely however, we found an interesting fact: acid secretion of one group of patients (group C) increased with a simultaneous decrease in serum gastrin. These changes in response to *H. pylori* eradication were similar to those observed in GU patients. Therefore, we divided DU patients into three groups according to changes in MAO by *H. pylori* eradication (group A: MAO decreased more than 1 mEq/h; group B: no change; group C: MAO increased more than 1 mEq/h) and found that group C patients had the lowest serum pepsinogen (PG) I levels and PG I/II ratios among the three groups (PG I: group C 44.7 (1.6), group A 64.5 (2.5), group B 57.8 (2.6); PG I/II: group C 2.52 (0.18), group A 3.98 (0.24), group B 3.60 (0.19); $p < 0.05$). These data indicate that patients in group C had suffered atrophy of the fundic mucosa.

Data on changes in gastrin stimulated acid secretion after eradication in patients with DU are controversial,²⁻⁵ although most reports show a decrease in acid secretion.^{2,3} As Iijima *et al* indicated, the most likely reason for the controversy may be that a considerable number of patients with DU had corpus gastritis, which may somehow cause hypoacidity. We previously reported that gastric acid secretion in Japanese subjects is lower than that in Europeans or North Americans, irrespective of *H. pylori* infection.⁶ As *H. pylori* is reported to induce corpus gastritis more easily in subjects with decreased acid secretion,^{7,8} we suggested that innate low gastric acid secretion of the Japanese may be responsible for the higher incidence of corpus gastritis and atrophy in Japanese subjects with *H. pylori* infection. This appears to be the case even for patients with DU in Japan, and it may be the reason why acid secretion was not significantly reduced after *H. pylori* eradication in our DU patients.

Figure 2 Correlation between decrease in serum gastrin concentration ($-\Delta$ Gastrin) and increase in maximum acid output (Δ MAO) in response to pentagastrin (6 µg) by *Helicobacter pylori* eradication in Japanese patients with duodenal (DU) and gastric (GU) ulcers. Groups A, B, and C show DU patients in whom MAO decreased (more than 1 mEq/h), did not change, and increased (more than 1 mEq/h) after eradication, respectively.