Relationship of Anti-Lewis x and Anti-Lewis y Antibodies in Serum Samples from Gastric Cancer and Chronic Gastritis Patients to Helicobacter pylori-Mediated Autoimmunity

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Lewis (Le) antigens have been implicated in the pathogenesis of atrophic gastritis and gastric cancer in the setting of Helicobacter pylori infection, and H. pylori-induced anti-Le antibodies have been described that cross-react with the gastric mucosa of both mice and humans. The aim of this study was to examine the presence of anti-Le antibodies in patients with H. pylori infection and gastric cancer and to examine the relationships between anti-Le antibody production, bacterial Le expression, gastric histopathology, and host Le erythrocyte phenotype. Anti-Le antibody production and H. pylori Le expression were determined by enzyme-linked immunosorbent assay, erythrocyte Le phenotype was examined by agglutination assays, and histology was scored blindly. Significant levels of anti-Leα antibody (P < 0.0001, T = 76.4, DF = 5) and anti-Leβ antibody (P < 0.0001, T = 73.05, DF = 5) were found in the sera of patients with gastric cancer and other H. pylori-associated pathology compared with H. pylori-negative controls. Following incubation of patient sera with synthetic Le glycoconjugates, anti-Leα and -Leβ autoantibody binding was abolished. The degree of the anti-Leα and -Leβ antibody response was unrelated to the host Le phenotype but was significantly associated with the bacterial expression of Leα (r = 0.863, r² = 0.745, P < 0.0001) and Leβ (r = 0.796, r² = 0.634, P < 0.0001), respectively. Collectively, these data suggest that anti-Le antibodies are present in most patients with H. pylori infection, including those with gastric cancer, that variability exists in the strength of the anti-Le response, and that this response is independent of the host Le phenotype but related to the bacterial Le phenotype.

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proven gastric cancer from a geographical area of high H. pylori occurrence to determine the prevalence of anti-Le\textsuperscript{a} and Le\textsuperscript{b} antibodies in vivo in patients with gastric cancer, thereby testing the hypothesis that anti-Le\textsuperscript{a} and anti-Le\textsuperscript{b} antibodies may play a role in H. pylori-mediated autoimmunity and in gastric cancer pathogenesis. The role of host Le antigen phenotype was also evaluated with respect to the strength of the anti-Le antibody response in our patient groups.

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MATERIALS AND METHODS

Patients. Subjects were recruited from a group of 207 consecutive persons attending the open-access endoscopy service at University College Hospital, Galway, Ireland, and included 120 men and 87 women (mean age, 54 years; range, 13 to 90 years). Persons who had received antibiotics, H2 receptor antagonists, or proton pump inhibitors during the 4 weeks prior to upper gastrointestinal endoscopy were excluded. All patients were Irish, and all were Caucasian. Serum was also available from 48 consecutive subjects with gastric cancer from an area of high prevalence of H. pylori infection (Kaunas, Lithuania). All patients gave informed consent for inclusion in this study.

Specimens and analysis. For Irish patients, during upper endoscopy three gastric antral biopsy specimens were obtained using the same size biopsy forceps from similar topographic sites at each endoscopy from within 3 cm of the pylorus. Fundal biopsies were obtained only when endoscopic examination suggested gastric atrophy. Blood samples (10 ml) were collected into tubes containing EDTA and clot activator at the time of endoscopy from a single venepuncture site immediately prior to the administration of sedation.

One of the antral biopsy specimens was cultured for H. pylori, and confirmatory biochemical and microscopic tests were performed (39). Stock cultures were maintained at -70°C in horse serum supplemented with 20% glycerol and, when required for characterization, H. pylori isolates were cultured on blood agar for 48 h and identified as described above. A second biopsy was smeared on a glass slide and examined for H. pylori using a Giemsa stain. Sections of the third biopsy specimen embedded in paraffin wax were prepared and stained with hematoxylin and eosin stain for light microscopy as described previously (19) and assessed subjectively by one blinded histopathologist for H. pylori colonization density. Sections were graded 0 to 3, corresponding, respectively, to absent, scant, moderate, and heavy bacterial colonization. The severity and activity of gastritis in the same specimens were also graded 0 to 3, according to the criteria described previously (19). For all patients, antibodies (immunoglobulin G) against H. pylori were measured in patient sera by a qualitative enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (HM-CAP; Sigma, St. Louis, Mo.), according to the manufacturer's instructions. H. pylori infection was defined as being present if the culture alone or any combination of two other tests was positive (18, 19).

Determination of H. pylori blood group antigen phenotype. Cells of H. pylori isolates were harvested from blood agar plates and prepared as described previously (18). Protein concentrations of cell suspensions were measured in patient sera by a qualitative enzyme-linked immunosorbent assay, and the optical density values measured at 492 nm (OD\textsubscript{492}) were considered positive for the presence of blood group antigens if the OD was greater than 0.3 OD\textsubscript{492} units (ODU), since nonspecific binding never exceeded this value.

Determination of the presence of anti-Le\textsuperscript{a} and anti-Le\textsuperscript{b} antibodies in serum. To determine whether or not antibodies to Le\textsuperscript{a} and/or Le\textsuperscript{b} were present in patient sera, experiments were performed in which patient serum was incubated overnight with LPS, bacterial whole cells, and Le glycoconjugates to evaluate whether addition of patient serum reduced binding of the commercial anti-Le\textsuperscript{a} and anti-Le\textsuperscript{b} antibodies. For experiments using bacterial whole cells, preparations of H. pylori NCTC 11637 and P466 were prepared as described above. Flat-bottom microtiter plates were coated overnight with 100 μl of twofold dilutions of H. pylori cell suspensions in bicarbonate coating buffer (40). Twofold dilutions of patient serum (100 μl of phosphate-buffered saline [PBS] plus 1% fetal calf serum plus patient serum) were added and incubated overnight at 37°C, blocked with 2% BSA for 2 h and, subsequently, incubated with twofold dilutions of anti-Le mouse MAb in PBS containing 1% BSA and 0.1% Tween 20 (PBS-BSA-Tween) at 37°C for 2 h. Between incubation steps, wells were washed three times with PBS-Tween. Specificity of MAb binding was defined as in the previous experiments. Bound mouse antibodies were detected using goat anti-mouse antibodies conjugated with horseradish peroxidase (Sigma). Thus, inhibition of the binding of commercially available anti-Le\textsuperscript{a} and anti-Le\textsuperscript{b} antibodies by patient sera was measured. Control experiments were performed as before.

Experiments with glycoconjugates and purified LPS were conducted in a similar manner but with 10 μg of Lea-BSA or Lea-HSA and also purified LPS from H. pylori strains NCTC 11637, P466, and MO19 coated onto microtiter plates overnight in bicarbonate coating buffer.

Absorption experiments. To determine whether the binding of patient sera that had reacted with Le\textsuperscript{a} and Le\textsuperscript{b} epitopes in the previous ELISA experiments could be reduced or removed, sera were pretreated with incubation with glycoconjugates of Lea-BSA or Lea-HSA at room temperature overnight before testing. Absorbed sera were added in the ELISA experiments, and the reduction in inhibition of binding of commercially available anti-Le\textsuperscript{a} and anti-Le\textsuperscript{b} antibodies was measured.

Host Le blood group antigen typing. Host Le antigen phenotypes were determined with a macroscopic tube agglutination technique on washed erythrocytes, within 24 h of collection, using commercially available murine anti-Le\textsuperscript{a} and anti-Le\textsuperscript{b} blood grouping reagents (Bioscot Labs, Edinburgh, Scotland) in accordance with the manufacturer's instructions.

Statistical analysis. For quantitative data, analysis was performed using the Mann-Whitney and Kruskal-Wallis tests for comparisons of two, and more than two, independent groups, respectively. The colonization density of H. pylori and the lymphocyte and neutrophil densities were expressed as the means ± the standard error of the mean. Correlations were performed between continuous variables using either linear regression with Pearson's product moment correlation or by calculation of Spearman's rank correlation coefficient (r\textsubscript{s}). Significance was set at the 5% level (two-tailed P). All data analysis was performed using StatsDirect statistical software package, version 1.7.3.

RESULTS

Patients, H. pylori infection, and pathologies. Of the 207 patients undergoing endoscopy, H. pylori isolates were available for analysis in 84 (49 men and 35 women; mean age, 51.2 years; range, 17 to 90 years). Of these, 78 (93%) had endoscopically visible disease. Twelve patients (14%) showed duodenal ulceration, and ten (12%) showed gastric ulceration, all in conjunction with antral gastritis. Fifty-six patients (67%) had endoscopic evidence of chronic gastritis, and six patients (7%) had normal endoscopic findings. These patients were classified as having nonulcer dyspepsia after sonography of the upper abdomen was found to be normal. Of the Irish patients who were H. pylori negative (n = 123) by our defined criteria (culture negative, histology negative, serology negative), serum was examined from 60 of these patients for the presence of anti-Le antibodies. This group is referred to as the control group in the remainder of this study. All patients with gastric cancer were H. pylori positive.

Presence of anti-Le\textsuperscript{a} and anti-Le\textsuperscript{b} antibodies in sera of patients with H. pylori-related pathologies. ELISA experiments were undertaken using whole cells of H. pylori, glycoconjugates of Le antigens, or H. pylori LPS to determine whether or not anti-Le antibodies were present in the serum of our patient groups. Results of the greatest consistency were obtained when purified LPS was coated onto the ELISA plates and, therefore, this methodology was used throughout the study. In particular,
in experiments in which glycoconjugate-coated ELISA plates were used, consistent results were obtained in only about 50% of tests, suggesting that synthetic glycoconjugates were inefficiently bound within the experimental system or that antigen presentation by glycoconjugates was suboptimal and variable between experiments. Consistent with the latter suggestion, it has been shown previously (4) that bound glycoconjugates of monomeric Le\(^x\) or Le\(^y\) were less effective at detecting anti-Le antibodies in \textit{H. pylori}-positive sera than were bound polymeric Le antigens similar to those found in \textit{H. pylori} LPS. In addition, a comparative study of the binding of anti-Le antibodies to soluble and plate-coated glycoconjugates was performed to assess the influence of soluble versus solid-phase presentation of antigen for anti-Le antibody recognition. This validation study found greater and more consistent antibody binding to soluble (100%) than to the plate-bound Le glycoconjugates.

Binding of commercially available anti-Le\(^x\) antibody to the LPS of \textit{H. pylori} NCTC 11637 (Fig. 1A), which expresses Le\(^x\), was measured at a median of 1.45 ODU (range, 1.42 to 1.48 ODU). When patient sera were incubated with the LPS of NCTC 11637, a significant reduction in binding of commercial anti-Le\(^x\) antibody was found for patients with gastric cancer, duodenal ulceration, chronic gastritis, and gastric ulceration compared with the control group that was \textit{H. pylori} negative (\(P < 0.0001, T = 76.4, DF = 5\), Kruskal-Wallis test). To confirm that the observed immune responses were related to Le\(^x\) determinants on the O-side chains of LPS alone and not due to reaction with other areas within \textit{H. pylori} LPS, such as the core region or lipid A region, absorption experiments were performed. After absorption of the patient sera with synthetic Le\(^x\) glycoconjugate, binding of the commercial anti-Le\(^x\) antibody was enhanced significantly for all disease groups (Fig. 1B). The ODU readings from all patient groups fell within the range observed for the patients who were \textit{H. pylori} negative, and no statistically significant difference (\(P > 0.05\)) was found between groups for binding of the anti-Le\(^x\) antibody.

In a similar manner, direct binding of commercial anti-Le\(^y\) antibody to the LPS of \textit{H. pylori} P466 (Fig. 2A), which expresses Le\(^y\), measured 1.485 ODU (range, 1.43 to 1.5 ODU). When patient sera were incubated with the P466 LPS, a significant reduction in binding of anti-Le\(^y\) antibody was again found in patients with gastric cancer, duodenal ulceration, chronic gastritis, and gastric ulceration compared to \textit{H. pylori}-negative controls. Also, the difference in antibody binding between patients with nonulcer dyspepsia and controls was significant (\(P < 0.0001, T = 73.05, DF = 5\), Kruskal-Wallis test). Absorption of patient sera with synthetic Le\(^y\) glycoconjugate was performed and, excluding the control group, a significant enhancement of binding of the commercially available anti-Le\(^y\) antibody was found when absorbed patient sera were tested (Fig. 2B). The ODU readings from all patient groups fell within the range observed for the \textit{H. pylori}-negative controls, and no statistically significant difference (\(P > 0.05\)) was found between groups for binding of the anti-Le\(^y\) antibody.

Moreover, for both anti-Le\(^x\) and anti-Le\(^y\) antibodies, and in all disease groups, a range of inhibition of commercial anti-Le antibody binding was observed (Fig. 1A and 2A), suggesting that the potency of the anti-Le response varies between individual patients.

**Relationship of bacterial Le expression to anti-Le\(^x\) and anti-Le\(^y\) antibodies.** Eighty-eight percent (74 of 84) of \textit{H. pylori} isolates were identified as having Le\(^x\), Le\(^y\), or a combination of Le\(^x\) and Le\(^y\) expressed on their LPS. This total included 4 strains (5%) with Le\(^x\) alone, 23 strains (27%) with Le\(^y\) alone, and 47 strains (56%) expressing both Le\(^x\) and Le\(^y\). Ten isolates (12%) had no evidence of either Le\(^x\) or Le\(^y\) expression on their LPS. Figure 3 shows the relationships between the presence of anti-Le\(^x\) and anti-Le\(^y\) antibodies in patient sera, and the expression of Le\(^x\) and Le\(^y\) on the \textit{H. pylori} isolates from the same patients. The ODU values obtained for the amount of Le\(^x\) and Le\(^y\) on individual isolates is plotted against the ODU values of inhibition of binding of commercial anti-Le\(^x\) or -Le\(^y\) antibody, respectively, to \textit{H. pylori} LPS by patient sera. Using Pearson’s
and Leα in nonsecretors or Leβ and Leε in secretors and, hence, it was of interest to determine whether a relationship existed between host Le phenotype and anti-Le responses. Of the 84 H. pylori-positive patients, 58 (69%) were secretors [Le(a−,b+)], 21 (25%) were nonsecretors [Le(a+,b−)], and 5 (6%) had the recessive [Le(a−,b−)] phenotype. Since secretor status cannot be determined from the recessive [Le(a−,b−)] phenotype and since salivary testing is required, patients with the recessive phenotype were excluded from further analysis. Random analysis of gastric biopsies for Le antigen expression gave consistent results with those of erythrocyte typing. Patients who expressed Leα on their erythrocytes or epithelial surfaces (nonsecretors) were not more likely to have higher levels of anti-Leα antibody present in their sera than secretors who expressed Leα (1.147 ± 0.187 ODU versus 1.16 ± 0.177 ODU; P = 0.677, Mann-Whitney test) and, also, secretors did not produce a significantly higher level of anti-Leα LPS antibody than nonsecretors (1.117 ± 0.162 ODU versus 1.155 ± 0.175 ODU; P = 0.524, Mann-Whitney test).

Histological findings and anti-Leα and anti-Leε antibody production. Using Spearman’s correlation coefficient, we examined the relationships between anti-Leα and anti-Leε antibody production and bacterial colonization density, as well as lymphocyte and neutrophil inflammatory responses in patients classified into two groups: those with peptic ulceration and those with chronic gastritis alone, excluding patients with nonulcer dyspepsia (Table 1). For both groups, no statistically significant association (P > 0.05) was identified between anti-Leα and anti-Leε antibody production and any of the histological parameters. However, a trend toward significance (r = 0.407, P = 0.060) was observed between the antral lymphocyte infiltrate and the anti-Leα antibody response in the cohort of patients with peptic ulceration. A similar, but less significant, trend (r = 0.216, P = 0.108) was noted between the observed lymphocyte infiltrate and the anti-Leε response in the cohort of patients with chronic gastritis.

**DISCUSSION**

The results of the present study provide evidence for a relationship between Leα and Leε antigen expression on H. pylori isolates and the development of anti-Le induced antibodies in their infected hosts. In addition, we have identified anti-Leα and anti-Leε antibodies in the sera of patients with gastric cancer. Sera from H. pylori-infected patients reacted much more strongly with the LPS of H. pylori NCTC 11637 and P466, expressing polymeric Leα and polymeric Leε with terminal monomeric Leγ (5–7), respectively, than sera from noninfected controls (Fig. 1 and 2). These experiments also demonstrated for the first time that not only do antibodies found in the serum of patients infected with H. pylori cross-react with LPS but these antibodies also recognize Le epitopes within LPS. It has been suggested previously that a major fraction of the human serum response to the LPS of H. pylori-infected individuals might be directed toward the LPS core or lipid A regions (4) or non-Le epitopes present in the O-polysaccharide chain of LPS (43, 44). The results of the present study clearly show the reaction of human sera with Le epitopes within LPS; first, because of the methodology of the inhibition ELISA which determined binding of commercial anti-Leα or -Leε antibodies to

![A](https://example.com/imageA.png)

**FIG. 2.** Inhibition of binding of commercially available anti-Leα antibody to Leε-expressing H. pylori LPS (P466) by patient sera of different disease groups before absorption (A) and after absorption (B) with Leε glycoconjugate by patient sera of different disease groups. Direct binding of commercial anti-Leε alone to the LPS was measured (LPS), and the abilities of unabsorbed or absorbed sera from gastric cancer (Cancer), duodenal ulcer (DU), chronic gastritis (Gastr), gastric ulcer (GU), and nonulcer dyspepsia (NUD) patients, as well as from a control group (Cont), to inhibit binding of the commercial antibody were determined. Excluding the control group, a significant enhancement of binding of the commercially available anti-Leε antibody was found when absorbed patient sera were tested.

PRODUCT MOMENT CORRELATION, A HIGHLY SIGNIFICANT CORRELATION was found between the presence of Leα determinants on H. pylori isolates and the presence of anti-Leα antibody (Fig. 3A; r = 0.863, r² = 0.745, P < 0.0001) and bacterial expression of Leε determinants and the presence of anti-Leε antibody in patient sera (Fig. 3B; r = 0.796, r² = 0.634, P < 0.0001).

Relationship of host Le phenotype to anti-Leε and anti-Leγ responses. Lewis blood typing can identify both the Le antigen phenotype and the secretor status of most individuals. Leα is the predominant blood group antigen expressed on epithelial cell surfaces and the erythrocytes of secretors, whereas Leε is that expressed by nonsecretors, but expression of both type 1 and type 2 Le antigens occurs in the gastric mucosa (22, 33). In particular, surface and foveolar epithelia coexpress either Leα and Leε. It has been suggested previously that a major fraction of the human serum response to the LPS of H. pylori-infected individuals might be directed toward the LPS core or lipid A regions (4) or non-Le epitopes present in the O-polysaccharide chain of LPS (43, 44). The results of the present study clearly show the reaction of human sera with Le epitopes within LPS; first, because of the methodology of the inhibition ELISA which determined binding of commercial anti-Leα or -Leε antibodies to

![B](https://example.com/imageB.png)
LPS after reaction with patient sera (Fig. 1A and 2A) and, second, by the reduction of inhibition within the ELISA of anti-Le autoantibodies when patient sera were preincubated with synthetic Le glycoconjugates in absorption experiments (Fig. 1B and 2B).

That the experimental system performed at its optimal when LPS, rather than whole cells or synthetic glyconjugates, was coated onto reaction plates is also noteworthy, since it demonstrates that antigen presentation may be of importance in the recognition of an epitope by an autoreactive antibody, as suggested previously (25). In particular, glycoconjugate-coated ELISA plates yielded consistent results in only about 50% of tests compared to LPS-coated plates, and a comparative analysis of anti-Le antibody binding to soluble and plate-bound Le glycoconjugates showed greater and more consistent antibody binding to the former antigen format. Moreover, a previous comparative study of serological assays using different antigen presentation formats has shown differences in ability to detect anti-Le\(^x\) and anti-Le\(^y\) antibodies in \textit{H. pylori}-positive patient sera (20). Although glycoconjugate-coated ELISA plates have been applied previously to detect anti-Le antibodies in \textit{H. pylori} patient sera and could not effectively demonstrate differences compared to control sera (1), those studies which have used ELISA with LPS or cell-derived preparations were able to

FIG. 3. Correlation of inhibition of binding of commercial anti-Le\(^x\) (A) and anti-Le\(^y\) (B) antibodies by the sera of \textit{H. pylori}-positive patients with bacterial expression of Le\(^x\) and Le\(^y\), respectively, on isolates from the same patients.
show differences between infected and noninfected individuals (4, 34), again reflecting the importance of the antigen presentation format. Consistent with this interpretation, the commercially available synthetic Le glycoconjugates that were used in the present and previous (42) studies contained monomeric Le antigens only but, in a small study, plate-coated glycoconjugates of monomeric Le or Le were found to be less effective in binding anti-Le antibodies in H. pylori-positive sera than were natural polymeric Le antigens (4), which were similar to those found in H. pylori LPS (5–7, 23).

Yokota et al. (42) have reported in their study of Japanese H. pylori isolates that the antigenicity of the O-polysaccharide chain varied depending on the strain and disease group; particularly, those from gastric tumors showed low antigenicity. Moreover, in the present study, the potency of the anti-Le response varied between individual patients, possibly reflecting these differences in antigenicity but also reflecting the immune responsiveness of the host. In further studies, Yokota et al. (43, 44) reported the occurrence of a highly antigenic epitope and a weakly antigenic epitope, both expressed in the O-polysaccharide of H. pylori, as well as the occurrence of strains not expressing an O-polysaccharide chain (rough-LPS). It is important to note that subculturing of strains in vitro can lead to loss of O chains from H. pylori LPS (27), but in this study isolates were handled to maintain Le antigen expression under the conditions optimized in our previous investigations (18, 19, 39). Although Yokota et al. were unable to correlate the epitopes in the O chains with the presence of Le antigens by the methods they employed (43, 44), the sugar composition of the LPS they analyzed was consistent with the presence of Le antigens (44). Furthermore, attempts by these workers to produce MAbs and rabbit antisera against epitopes independent of Le antigens have failed to date (44), and only anti-Le antibodies have been obtained. Therefore, this raises questions to the identity of the epitopes identified by Yokota et al. (43, 44) and their relationship to Le antigens.

In this study we have determined that the principal determinant of the anti-Le response is not related to host Le phenotype per se but rather to the amount of bacterial Le and Le expression. Highly significant correlations were found between the presence of bacterial Le determinants and the presence of anti-Le antibody in patient sera (Fig. 3A; $r^2 = 0.745$). Similar, but not as significant, was the correlation between the presence of bacterial Le determinants and anti-Le in patient serum (Fig. 3B; $r^2 = 0.634$). These results contrast with those of one study in which absorption experiments with H. pylori to remove autoantibody reactivity against the parietal cells of the gastric mucosa and anti-Le antibodies were unsuccessful (14) but are in agreement with those of another study in which autoantibodies were diminished by absorption with Le-positive H. pylori lysates (30). Moreover, Negri et al. reported that sera from 84% of patients with H. pylori infection reacted with antral gastric mucosa and 66% of patient sera with corpus gastric mucosa (30, 31) and that the prevalence of anti-Le autoantibodies correlated with lymphocytic infiltrate and gastric gland atrophy (31). Consistent with these findings, anti-Le H. pylori-induced autoreactive antibodies in mice can react with the β chain of the H+-K+-ATPase gastric proton pump (4) but, in the light of the failed absorption experiments with human sera in one study (14), these results were reinterpreted to indicate differences between mouse and human autoantibodies (2).

However, the results of the present study show that this interpretation should be reconsidered. The discrepancies between the results of absorption studies (14, 30) and the inability to detect anti-Le antibodies in patient sera (9, 25) may reflect the importance of antigen presentation in the test system. First, in the present study, the LPS of H. pylori P466 (which expresses monomeric Le carried on polymeric Le) was used in the detection of anti-Le antibodies rather than a monomeric antigen format. Second, the outer membrane of H. pylori undergoes blebbing, thereby producing outer membrane vesicles (OMVs) which contain LPS expressing Le antigens (15, 21, 25). Interestingly, absorption of patient sera from the present study with synthetic glycoconjugates and OMVs, under the same conditions as used in this study, has yielded preliminary results showing a decrease in their autoreactivity with human gastric mucosa (A. P. Moran et al., unpublished data). Differences in the presentation of Le antigens on the OMV surface versus that on the bacterial surface may also explain the difficulties encountered in absorbing anti-Le antibodies from patient sera with bacterial whole-cell lysates in a previous study (14), which led to the conclusion that the production of anti-Le antibodies was independent of bacterial Le expression (9, 14).

We have previously reported that bacterial Le expression in patients with H. pylori-related ulcer disease was significantly related to lymphocyte infiltration of the gastric mucosa (18). In particular, in patients with chronic gastritis, significant relationships were found between the expression of Le and H. pyl-
lori colonization density and neutrophil and lymphocyte infiltrates, and bacterial Le^\text{e}\textsuperscript{+} expression was related to neutrophil and lymphocyte infiltration. In other studies, experiments indicate that neutrophils are a potential target recognized by anti-Le^\text{e}\textsuperscript{+} antibodies (4, 35). Neutrophils express CD15 (Le^\text{e}) on members of the adhesion promoting glycoprotein family (CD11/CD18). Anti-Le^\text{e} MAbs activate and cause enhanced adherence of these cells, which may result in tissue damage and inflammation (4, 35, 36). Also, recent data indicate that Le antigens may play a role in bacterial adhesion to the gastric mucosa (12), thereby aiding the contact of secreted products with the mucosa and thus potentiating the development of the inflammatory response (25). In this study, no significant association was observed between the presence of a potentially autoreactive anti-Le antibody and any histological parameter, although a trend (r^2 = 0.407, P = 0.060) was observed between antral lymphocyte infiltrate and the anti-Le^\text{e} response in patients with peptic ulceration. A less significant trend (r^2 = 0.216, P = 0.108) was noted between the observed lymphocyte infiltrate and the anti-Le^\text{e} response in the cohort of patients with chronic gastritis. That the predominant inflammatory infiltrates in this group of patients in the setting of anti-Le antibodies are lymphocytes is, in our opinion, unexpected. Acute inflammatory cells occurring as the predominant cell type would be more likely to be early after colonization with H. pylori, rather than late as in the setting of chronic gastritis. This concept is supported by the fact that the presence of anti-Le antibodies is likely to be determined by the duration of infection with H. pylori (8).

It is unlikely that the presence of anti-Le^\text{e} and anti-Le^\text{h} antibodies in H. pylori-positive patients would be solely responsible for both the initiation and the maintenance of an autoimmune response in infected patients. Nevertheless, based upon the available data, it can be speculated that the mechanisms underlying autoantibody production likely include molecular mimicry between constitutively expressed bacterial LPS and those of the gastric mucosa (4, 25, 26, 30, 31), as well as facilitated exposition of the bacterial epitopes to a recruited gastric mucosal immune population which is influenced by host immune regulation and environmental factors. Subsequently, host structural epitopes could become exposed and presented to the immune system to further drive the autoreactive response, as suggested previously (2, 14). A cross-reactive antibody such as anti-Le^\text{e} may initiate damage to the proton pump, with subsequent alteration in acid output (4). Changes in acid output can influence the LPS structure of the infecting bacterial strain such that phase variation occurs in the generation of Le epitopes (28) and, hence, a secondary reduction in autoreactive antibody production could occur, thereby confounding interpretation of the role of H. pylori-induced anti-Le antibodies. Moreover, anti-Le^\text{e} antibodies may augment the immune response by causing complement-mediated lysis of host target cells as observed in other infections (32, 37). Following the initiation of the inflammatory cascade, other virulence factors, such as those associated with the cagA and vacA genes, would maintain the immune response with consequent progressive histological damage (24). Although the relationship between the presence of anti-Le antibodies and disease states is not clear, utilization of transgenic mice models expressing Le determinants (13, 17), coupled with longitudinal studies in human beings known to express clonal isolates of H. pylori (41), may help clarify the full significance of anti-Le autoimmunity.

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