Introduction

Blood group antigens (Lewis and ABH antigens) are carbohydrate structures originally identified on RBC by Landsteiner. However, these antigens are widely expressed in many tissues throughout the body, being especially abundant in the epithelial cells of gastric mucosa. The secretor status is defined by the presence of ABH antigens in body fluids and secretions like saliva, gastric juice, and milk.

Lewis (Lewis<sup>a</sup>, Lewis<sup>b</sup>) and ABH antigens are closely interrelated, and in mucosecretory epithelia they are produced from a common precursor (type 1 precursor) by the action of different genes. In the gastric foveolar cells, the expression of Lewis and ABH antigens is fundamentally controlled by the action of the secretor and Lewis genes (Fig. 1). In people who have the secretor and Lewis genes, all of the precursor substance is transformed into H type 1 antigen, and they express Lewis<sup>b</sup> and ABH antigens in all of the epithelial cell layers of the stomach. In people who do not have the Lewis gene, all of the precursor substance is transformed into Lewis<sup>a</sup> antigen, finally, people who do not have both the secretor and Lewis genes do not express Lewis<sup>a</sup> or Lewis<sup>b</sup> antigens.

Materials and Methods

Subjects in this study were selected from a randomized double blind, placebo-controlled chemoprevention trial, currently being conducted in Tachira State, Venezuela, the aim of which is to assess the effect of antioxidant vitamins on progression of precancerous lesions of the stomach. The results presented here are a cross-sectional analysis of the subjects who had a histological diagnosis of IM at baseline. Although follow-up data are available, analysis of these data has been deferred until the randomization code is broken and the cohort of subjects receiving placebo can be identified.

To explore the role of the alterations in the expression of Lewis antigens in the gastric precancerous process, we have studied histochemical and antigenic anomalies on gastric biopsies from a population from the Andean region in Venezuela with high risk for gastric cancer. We have correlated the Lewis and secretor phenotypes with histological lesions and with Helicobacter pylori infection. The expression of anomalous Lewis<sup>a</sup> antigen in relation to type I Lewis<sup>a</sup>b gastric lesions was studied.

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The abbreviation used is: IM, intestinal metaplasia.
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curvature of the antrum, ~1 cm above the pylorus; one from a midportion of the lesser curvature of the antrum; one from the lesser curvature of the antrum immediately below the incisura; and one from the middle corpus, ~2 cm from the lesser curvature.

Gastric biopsies were fixed in buffered formalin and stained with H&E and Giemsa to detect H. pylori. The most severe lesion among all biopsies was considered for the global diagnosis and for the immunohistochemical studies. Those biopsies positive for IM were also stained with periodic acid-Schiff-Alcian blue (6) and high iron diamine-Alician blue (7) to determine subtypes of IM that were classified according to the method of Filipe and Jass (8) as follows. Type I is characterized by the presence of mature goblet and absorptive cells with a well-defined brush border. Goblet cells secrete sialomucins. In type II, there is mild architectural distortion, crypts are lined by goblet cells, absorptive cells are few or absent, and columnar mucous cells are present containing a mixture of neutral and acid sialomucins. The goblet cells secrete sialomucins or occasionally sulfomucins, or both. In type III, the overall architecture is more disorganized than in type II. Columnar cells secrete sulfomucins, and goblet cells contain sialo- or sulfomucins.

Lewis and secretor phenotypes were determined by immunohistochemistry in nonmetaplastic areas of gastric mucosa, as previously reported (9). Anti-A (A581, Dako; working dilution, 1:40), anti-B (A582, Dako; working dilution, 1:40), and anti-H type 2 (A583, Dako; working dilution, 1:40) were used to determine the secretor phenotype. The presence of ABH antigens in the foveolar epithelium defined the secretor phenotype. The secretor phenotype was identified in the present study by using a multiple logistic regression model (9).

Results

Gastric biopsies taken at baseline were studied from 564 subjects with a global diagnosis of IM or dysplasia. The most severe lesion among all biopsies was considered as the global diagnosis. The subjects ranged in age from 34 to 71 (mean, 51), and 269 were men and 295 women. The secretor status was determined in only 552 patients.

The prevalence of the different histological lesions was 58% for type I IM, 12% for type II, 13% for type III IM, and 17% for dysplasia. In dysplastic cases, 81% showed mild, 17% showed moderate, and 2% showed severe dysplasia. Table 1 shows the Lewis and secretor phenotype distribution by sex. There was no significant difference in the distribution of either phenotype by sex. A high proportion (93%) of Lewis (a+b-) phenotype subjects were secretors. The prevalence of Lewis (a+b-) and nonsecretor individuals was lower than that of the European population, a fact observed in other Andean populations (9).

Secretor Status and H. pylori Infection. Table 2 shows the distribution of histological diagnosis by secretor status. There is an excess of secretors in the group of subjects with IM-I, but overall there is no significant difference in the distribution of the histological diagnoses between secretors and nonsecretors (P = 0.16; test for trend, P = 0.10).

Table 3 shows the relationship between secretor status and H. pylori infection, rated on a four point scale (negative, difficult to find, easy to find, abundant). H. pylori infection is very common in this population: 94% of the participants in the chemoprevention trial are infected. There is no significant difference
this could not be related to between secretor status and severity of histological diagnosis, but Abnormal Expression of Lewis a in Lewis (a expression of Lewisa . The trends are all strongly significant (type III, and IM type II with an increasing degree of abnormal type I as a baseline, there is a clear increasing risk of dysplasia, IM 2 b (a 2 1)

Discussion

Table 1 Distribution of Lewis phenotype and secretor status by sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>Lewis phenotype</th>
<th>Secretor status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Le(a+b−)</td>
<td>Le(a−b+)</td>
</tr>
<tr>
<td>Male</td>
<td>31 (12)*</td>
<td>187 (71)</td>
</tr>
<tr>
<td>Female</td>
<td>29 (10)</td>
<td>217 (75)</td>
</tr>
<tr>
<td>Total</td>
<td>60 (11)</td>
<td>404 (73)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses, percentage.

Table 2 Distribution of histological diagnosis by secretor status

<table>
<thead>
<tr>
<th>Secretor status</th>
<th>Histological diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IM-I</td>
</tr>
<tr>
<td>Secretor</td>
<td>289 (59)*</td>
</tr>
<tr>
<td>Nonsecretor</td>
<td>30 (45)</td>
</tr>
<tr>
<td>Total</td>
<td>319 (58)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses, percentage.

Table 3 Extent of H. pylori infection (overall prevalence in subjects with complete biopsy information) by secretor status

<table>
<thead>
<tr>
<th>Secretor status</th>
<th>H. pylori (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Secretor (n = 452)</td>
<td>17</td>
</tr>
<tr>
<td>Nonsecretor (n = 62)</td>
<td>23</td>
</tr>
</tbody>
</table>

Numbers in parentheses, percentage.

between secretors and nonsecretors in degree of H. pylori infection (P = 0.18). Secretor status was also examined in relation to three markers of cellular or mucosal damage, atrophy, regenerative activity, and erosion or ulcer, which were rated on a four point scale for each biopsy (negative, light, moderate, and severe for atrophy and regenerative activity and negative, superficial, mucosal penetration, and submucosal penetration for erosion or ulcer). No relationship was found with any of these markers (tables not shown; P = 0.77 for atrophy, P = 0.42 for regenerative activity, and P = 0.57 for erosion or ulcer. Trend test results, P = 0.32, P = 0.94, P = 1.00, respectively).

In summary, there is weak evidence of a correlation between secretor status and severity of histological diagnosis, but this could not be related to H. pylori infection.

Abnormal Expression of Lewis a in Lewis (a−b+) Individuals, by Type of IM. Table 4 shows the relationship between abnormal expression of Lewis a antigen and histological diagnosis in Lewis (a−b+) individuals. This relationship is shown in Fig. 2. With IM type I as a baseline, there is a clear increasing risk of dysplasia. IM type III, and IM type II with an increasing degree of abnormal expression of Lewis a. The trends are all strongly significant (P < 0.001 for dysplasia, P < 0.001 for IM-III, and P = 0.002 for IM-II). The trend for IM-III is stronger than for IM-II (P = 0.02), but the trend for dysplasia is weaker than for IM-III.

These results were unchanged after controlling for the confounding effect of age, sex, years of education (as a marker of socioeconomic status), and smoking status, which have been previously identified as risk factors for advanced precancerous lesions in this population.

Discussion

The intestinal or epidemic type of gastric cancer is considered the end result of a multistep process in which multiple factors are involved. H. pylori infection, dietary factors, nitroso compounds, oxidative damage, and lack of antioxidant vitamins produce a series of changes in the gastric epithelium which include progressive grades of atrophy, IM, and dysplasia and, finally, a malignant transformation (11). In this dynamic process, progressive alterations in the mucin components and the expression of anomalous antigens may be also observed.

Alterations in the expression of blood group antigens have been extensively described in gastric cancer (12–15). It has been proposed that these anomalies are produced by blockages in the normal synthesis of these antigens, resulting in loss of some of these structures and the appearance of aberrant antigens. The anomalous expression of Lewis a antigen in lesions of gastric intestinal IM and dysplasia from Lewis (a−b+) individuals has been previously reported. In our experience, this abnormality has not been observed in the earlier lesions of chronic gastritis and gastric atrophy or in areas of normal gastric mucosa. Previous studies have demonstrated that the simultaneous expression of anomalous Lewis a antigen and sulfomucins indicates a greater risk of preneoplastic progression (9, 16).

Our results show an increasing frequency in the prevalence and grade of severity of the anomalous Lewis a antigen expression correlated to the severity of the histological changes and to the severity of the histochemical alterations (sialo- and sulfomucin expression). Thus, the most severe pattern of anomalous Lewis a antigen expression (pattern III) was more prevalent in dysplastic cases than in type II or type III intestinal metaplasia.

The role of H. pylori as the most important etiopathogenic factor in chronic gastritis is well documented. However, its direct involvement in more advanced lesions (gastric atrophy, IM, dysplasia, and cancer) is poorly understood. Recently, the Lewis b and H antigens have been described as the receptors responsible for the attachment of H. pylori to the gastric mucosa (17). The cytoplasm of the foveolar cells and the gastric mucus are very rich in these antigens, and this fact can explain the especial tropism of this bacterium for the gastric mucosa.

We think that in secretor individuals, the abundant presence of Lewis a and H antigens in the gastric mucus acts as a protective mechanism that traps bacteria and prevents their attachment to the gastric epithelium. This protective mechanism has been proposed as an adaptive response to infective microorganisms by Slomiany (18). In the present study, there is no relation-
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Acknowledgments

Negative 97 (73*) 13 (10) 6 (5) 17 (13) 133
Pattern I 77 (70) 14 (13) 7 (6) 12 (11) 110
Pattern II 31 (53) 8 (14) 8 (14) 12 (20) 59
Pattern III 32 (33) 16 (16) 22 (22) 28 (29) 98

Table 4  Histological diagnosis by degree of abnormal Lewis* expression in Lewis (a−b+) subjects

<table>
<thead>
<tr>
<th>Lewis alteration</th>
<th>Histological diagnosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IM-I</td>
<td>IM-II</td>
</tr>
<tr>
<td>Negative</td>
<td>97</td>
<td>13</td>
</tr>
<tr>
<td>Pattern I</td>
<td>77</td>
<td>14</td>
</tr>
<tr>
<td>Pattern II</td>
<td>31</td>
<td>8</td>
</tr>
<tr>
<td>Pattern III</td>
<td>32</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>237</td>
<td>51</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

Fig. 2. Odds ratios of IM-II, IM-III, and dysplasia, using IM-I as baseline, by pattern of abnormal Lewis* expression in Lewis (a−b+) subjects.

ship between H. pylori infection and secretor phenotype. This is not necessarily inconsistent with the theory that H. pylori is trapped in the gastric mucosa in secretor individuals. When rating the degree of H. pylori infection, it was not possible to distinguish between bacteria attached to cells and bacteria in mucus.

Previously, we have observed a lower epithelial damage, grade of atrophy, and a lower prevalence of sulfomucin expression in secretor individuals than in nonsecretors. In this study, there was no relationship between secretor status and atrophy, regenerative activity or erosion or ulcer.

A strong relationship between the expression of sulfomucins and nonsecretor status has been described in precursor lesions of gastric cancer (19) and also in Barrett’s esophagus and Barrett’s adenocarcinoma (20). In the present study, there is a tendency for nonsecretors to have more type III IM and dysplasia than secretors but, possibly due to the small number of nonsecretor individuals, the difference was not significant.

In summary, our results indicate that at the same time as the morphological changes that occur during the process of gastric carcinogenesis, another series of events occurs. Thus, the anomalous appearance of Lewis* and sulfomucins in areas of intestinal metaplasia appear to behave as immunohistological indicators of a greater severity of morphological lesions. Further follow-up studies are required to clarify the role of predictive markers of risk in precursor lesions of gastric cancer.

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References


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