Short Communication

Lewis, Secretor, and ABO Phenotypes, and Sulfomucin Expression in Gastric Intestinal Metaplasia[1]

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Abstract

The closely interrelated Lewis, secretor, and ABO phenotypes have long been linked to the risk of peptic ulcers and gastric cancer and may modulate the interaction between *Helicobacter pylori* and the gastric surface epithelium. We explored the association between the expression of sulfomucins in gastric intestinal metaplasia, a known marker of preneoplastic progression, and the expression of Lewis, secretor, and ABO phenotypes, in 523 subjects from Nariño, Colombia, and 856 subjects from northern Spain. In both study populations, Lewis (a+/b−) and nonsecretor phenotypes showed a significant positive association with the expression of sulfomucins (odds ratios, 2.4 and 2.6, respectively).

Introduction

Lewis and ABO, major human alloantigens, are structurally interrelated (1) and jointly referred to as "histo-blood group" antigens, given their expression in erythrocytes and multiple tissue locations (2), and as "onco-developmental" antigens for their role in differentiation and oncogenesis (3). They are more abundantly expressed in gastrointestinal epithelia and secretions than in most other tissue locations (1). The biological significance of this observation has not been clarified.

The secretor status is defined by the expression (secretor) or lack of expression (nonsecretor) of ABO antigens in body fluids and specific tissue locations such as the gastric surface epithelium (4), where the secretor status also controls the expression of the Lewis phenotype: nonsecretors express the Lewis (a+/b−) phenotype, and secretors express the Lewis (a−/b+) phenotype. Lewis (a−/b−) individuals do not express Lewis antigens, but still display ABO antigens with either a secretor or a nonsecretor pattern according to their secretor status (1).

In the present study, we explored the association of Lewis, secretor, and ABO phenotypes with the expression of sulfomucins in gastric intestinal metaplasia, in two populations with different frequencies of blood group phenotypes and at different risk of gastric cancer. This inquiry was prompted by the following preliminary observations: (*a*) sulfomucins are proven markers of preneoplastic progression in the stomach (5); (*b*) the expression of sulfomucins in columnar-type cells is a hallmark of intestinal metaplasia type III, incomplete, or colonic, and identifies an advanced stage in the gastric premalignant evolution (5); (*c*) the simultaneous expression of sulfomucins and aberrant Lewis[a] antigen is associated with a greater risk than the expression of either marker alone (6); and (*d*) Lewis, secretor, and ABO phenotypes are related to the risk of peptic ulcers and gastric cancer (7), and they may also affect the adherence of *Helicobacter pylori* to the gastric epithelium (8).

Materials and Methods

Subjects of this study were individuals from the general population of Nariño, Colombia, and consecutive patients referred for endoscopy in various communities of northern Spain. Colombian subjects were enrolled in a long-term study of gastric carcinogenesis. Spanish subjects were enrolled in a study of intestinal metaplasia involving regional hospitals of the Guipuzcoa, Oviedo, Soria, and Navarra provinces. These studies were approved by the Louisiana State University Medical School Institutional Review Board and parallel committees in Colombia and Spain. All participants gave informed, written consent.

Four gastric biopsies (one corporal, three antral) were obtained routinely, fixed in 10% buffered formalin, and embedded individually in paraffin. Only subjects with intestinal metaplasia documented by microscopic examination of H&E-stained sections were included in this study. The *H. pylori* status was evaluated with the modified Steiner stain (9). Sulfomucins were identified in areas of metaplasia with high-iron diamine Alcian blue stain (10), and evaluated as absent or present in goblet and nongoblet (columnar) cells (6). Lewis, secretor, and ABO phenotypes were determined by immunohistochemistry in nonmetaplastic gastric mucosa, as reported previously (6). Anti-Lewis[a] (7 LE, BioGenex, working dilution 1:250) and anti-Lewis[b] (2.25 LE, BioGenex, 1:250) were used to determine the Lewis phenotype. Anti-A (A581, DAKO Corp., 1:40), anti-B (A582, DAKO Corp., 1:40) and anti-H type 2 (A583, DAKO Corp., 1:40) were used to determine the ABO phenotype. Detection of ABO antigens in the surface epithelium defined the secretor phenotype; a negative reaction identified the nonsecretor phenotype.

Samples from Colombia were processed at Louisiana State University Medical Center in New Orleans. All samples from Spain were processed at Aránzazu Hospital in San Sebastián. Identical histochemistry and immunohistochemistry protocols were used in both study locations. Positive and negative con-

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Table 1 Percentage frequency of Lewis, secretor, and ABO phenotypes among individuals with intestinal metaplasia in Colombia and Spain

	Colombia (n = 523)	Spain (n = 856)
Lewis (a+/b−)	3.8	19.2
Lewis (a−/b+)	74.0	70.4
Lewis (a−/b−)	22.2	10.4
Nonsecretor	6.3	20.7
Secretor	93.7	79.3
O	68.6	43.1
A	20.5	47.5
B	9.6	6.2
AB	1.3	3.2

Table 2 Percentage prevalence of sulfomucins by blood group phenotype in Colombia and Spain

	n	Total positive	Goblet cells only	Columnar cells
Colombia				
Lewis (a+/b−)	20	90.0	60.0	30.0
Lewis (a−/b+)	387	57.4	38.0	19.4
Lewis (a−/b−)	116	56.9	37.9	19.0
Nonsecretor	33	84.9	57.6	27.3
Secretor	490	56.7	37.5	19.2
O	359	60.2	40.7	19.5
A	107	55.1	37.4	17.7
B	50	52.0	24.0	28.0
AB	7	71.4	71.4	0
Spain				
Lewis (a+/b−)	164	76.2	34.1	42.1
Lewis (a−/b+)	603	58.4	32.2	26.2
Lewis (a−/b−)	89	73.0	38.2	34.8
Nonsecretor	177	77.4	34.5	42.9
Secretor	679	59.6	38.8	26.8
O	369	69.6	36.6	33.1
A	407	57.5	29.5	28.0
B	53	62.3	34.0	28.3
AB	27	66.7	40.8	25.9

trols were shared by both laboratories. Each preparation was examined by two observers. Interobserver disagreements (less than 10%) were solved by joint review. Multiple logistic regression analysis was used to evaluate the association between the expression of Lewis, secretor, and ABO phenotypes with the prevalence of sulfomucins. ORs[3] and 95% CIs were adjusted by age, sex, and study location.

Results

Five hundred twenty-three subjects from Colombia and 856 patients from Spain were analyzed. Colombian subjects ranged in age from 29 to 69 years (mean 52.1), 51.8% were women, and 91.2% were *H. pylori*-positive. Spanish patients ranged in age from 18 to 64 years (mean 47.9), 16.7% were women, and 75.8% were *H. pylori* positive. The frequency of males and females in Spain closely reflects that of the local general endoscopy clinic population. Table 1 shows the frequency distributions for Lewis secretor and ABO phenotypes in both study populations.

Table 2 shows the prevalence of sulfomucin expression by blood group phenotypes in Colombia and Spain. In both populations, that prevalence was higher in nonsecretors and Lewis (a+/b−) individuals than in secretors and those expressing other Lewis phenotypes. Table 3 shows the corresponding OR and 95% CI. In Colombia, the difference was observed in subjects with and without expression of sulfomucins in columnar cells. In Spain, it was observed only in patients who expressed sulfomucins in columnar cells. In Colombia and Spain, the difference in the prevalence of sulfomucin expression between secretors and nonsecretors was essentially unchanged when the analysis was limited to Lewis (a−/b−) individuals (Table 4). Only minor differences were observed among individuals expressing different ABO phenotypes.[1]

Table 3 ORs and 95% CIs relating the expression of sulfomucins with Lewis (a+/b−) and nonsecretor phenotypes

	Colombia[a]	Spain[a]	Both populations[b]
Lewis (a+/b−)	6.6 (1.9–41.6)	2.1 (1.4–3.1)	2.4 (1.6–3.5)
Nonsecretor	4.2 (1.7–12.5)	2.3 (1.6–3.5)	2.6 (1.8–3.7)

[a] Adjusted by age and sex.
[b] Adjusted by age, sex, and study site.

Table 4 Percentage prevalence of sulfomucins among Lewis (a−/b−) subjects by secretor status in Colombia and Spain

	n	Total positive	Goblet cells only	Columnar cells
Colombia				
Nonsecretor	12	83.3	58.3	25.0
Secretor	104	53.9	35.6	18.3
Spain				
Nonsecretor	13	92.3	38.5	53.8
Secretor	76	69.8	38.2	31.6

Discussion

Our results show a strong association of sulfomucin expression with the Lewis (a+/b−) phenotype and nonsecretor status in two distinct populations. Given that the association with the nonsecretor status persisted when the analysis was limited to Lewis (a−/b−) subjects and the fact that all Lewis (a+/b−) individuals are also nonsecretors, it is likely that the actual risk determinant is secretor status rather than Lewis phenotype.

The nonsecretor status is known to carry an increased risk of gastric and duodenal ulcers; however, no systematic association has been found between the secretor status and gastric cancer (7). *H. pylori* infection, strongly associated with the risk of peptic ulcers and gastric cancer, was highly prevalent in both study populations. We have observed recently that, although the prevalence of *H. pylori* infection is essentially the same among secretors and nonsecretors, it causes greater epithelial damage in nonsecretors than in secretors.[4] Therefore, the high prevalence of sulfomucin-positive intestinal metaplasia among nonsecretors may be a manifestation of an increased susceptibility to the effects of the infection. Concomitantly or alternatively, the secretion of sulfomucins, by inhibiting *H. pylori*

[3] The abbreviations used are: OR, odds ratio; CI, confidence interval.

[4] B. Ruiz, J. Torrado, E. T. H. Fontham, T. Ramakrishnan, J. Garay, and P. Correa. Blood group phenotypes modulate the outcome of *Helicobacter pylori* infection, submitted for publication.

colonization of the gastric mucosa, may constitute an adaptive response to the infection, as proposed by Slomiany *et al.* (11).

The expression of sulfomucins in columnar cells is a hallmark of intestinal metaplasia type III, incomplete, or colonic type, and identifies an advanced stage in the gastric premalignant evolution (5). In Colombia, the association between nonsecretor status and sulfomucin expression was observed in subjects with and without expression of sulfomucins in columnar cells. In Spain, it was observed only in those with sulfomucin expression in columnar cells. Evidently, the link between the secretor status and sulfomucin expression occurs at an earlier stage of preneoplastic progression in Colombia than in Spain, a finding that may be related to an infection with *H. pylori* earlier in life, higher overall prevalence of infection, and higher gastric cancer rates observed in Colombia.

Although our findings verify the association between sulfomucin expression and the nonsecretor status in both study populations, the frequency of nonsecretors is very low in Nariño, although gastric cancer rates are higher there than in northern Spain. This paradox may be explained by the important contribution of environmental factors in locations such as Nariño, where gastric cancer reaches epidemic proportions (12).

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