Lack of Clinical Usefulness of Das-1 Monoclonal Antibody and Mucin Expression as Risk Markers of Gastric Carcinoma in Patients With Gastric Intestinal Metaplasia

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Key Words: Monoclonal antibody; Das-1; Gastric intestinal metaplasia; Gastric adenocarcinoma; Helicobacter pylori; Mucins

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Abstract

Our aim was to evaluate the usefulness of the monoclonal antibody Das-1 as a premalignant marker of gastric intestinal metaplasia (GIM) associated with gastric cancer and its association with mucin expression.

We evaluated Das-1 and mucin expression in 4 groups: 1 (n = 50), gastric carcinoma, paired samples of the cancer area and GIM away from the tumor; 2 (n = 25), gastric or duodenal ulcer with Helicobacter pylori infection with GIM and chronic gastritis; 3 (n = 25), H pylori–autoimmune chronic atrophic gastritis with GIM; and 4 (n = 25), H pylori–chronic gastritis without GIM.

Das-1 immunostaining was observed in 20 (40%) of 50 cases in cancer areas. The expression of Das-1 in GIM from group 1 cases away from the cancer area was different from that in GIM from nontumor cases (groups 2 and 3): 13 (26%) of 50 vs 2 (8%) and 0 (0%) of 25 (P = .004). There was no association between Das-1 and mucin expression.

Das-1 expression was associated with GIM from patients with gastric cancer. However, this relation was weaker than previously reported, precluding clinical usefulness as a premalignant marker of GIM.

Preventive strategies for gastric cancer should lead to diagnosis at an early stage. Gastric intestinal metaplasia (GIM) has been considered a premalignant lesion in the development of an intestinal-type gastric adenocarcinoma. GIM is quite common, occurring in up to one quarter of the general population in Western countries. A previous US study demonstrated that among consecutive patients undergoing endoscopy, 13% of patients at very low risk for gastric cancer (Caucasians) and 50% of higher risk groups (Hispanics and Blacks) demonstrated GIM when routine protocol-mapping biopsies of normal appearing mucosa were performed. The recommendation for screening and/or surveillance of this lesion would lead to a prohibitively large number of endoscopies, taking into account the unproven value of such strategy. Thus, the finding of a biomarker for the specific GIM associated with gastric cancer would be very valuable because its use might increase the detection of curable gastric cancer, which continues to be the second most frequent cause of cancer deaths worldwide.

Some experts have suggested that type III GIM (the incomplete or colonic form of GIM) is the specific subtype of GIM associated with an increased risk of developing an intestinal-type gastric adenocarcinoma. However, GIM subtyping using histologic and histochemical mucin staining techniques seems to be a highly subjective procedure.

Previous results by Mirza et al suggested that the monoclonal antibody Das-1 could be a risk marker of malignant transformation of gastric mucosa because its expression was strongly associated with gastric carcinoma and it was clinically useful to simplify and differentiate the phenotypes of GIM.

In this sense, reactivity to the antibody was 93% in GIM associated with gastric adenocarcinoma and 35% in GIM with...
chronic gastritis without gastric carcinoma. No reactivity was observed in normal gastric mucosa. Das-1 (formerly called 7E12, IgM isotype) was developed against a 40-kDa colonic epithelial protein. It was found to specifically recognize a colon epithelial protein larger than 200 kDa that complexes with a 40-kDa protein and acts as a chaperone to bring the 40-kDa protein to colonic epithelial surface.

Because there are not studies by other laboratories confirming the promising data of Mirza et al,9 the aim of the present study was to evaluate the usefulness of Das-1 as a premalignant marker of the GIM associated with gastric cancer. In addition, Das-1 expression in the subtypes of GIM was evaluated, following standard histologic criteria and immunohistochemical techniques for mucin expression.

Materials and Methods

Cases

We used the database of the pathology department to randomly select paraffin-embedded tissue blocks from a total of 125 adult patients, distributed in the following groups: 1 (n = 50), surgical specimens from patients with a diagnosis of GIM associated with gastric carcinoma, of a total number of 366 gastric carcinomas seen in our unit during the 1999-2005 period; for each selected case, paired samples of stomach (surgical specimens) were evaluated, including the cancer area and the histologically proved GIM area away from the tumor; 2 (n = 25), gastric biopsy specimens from the antral mucosa obtained from patients with a gastric or duodenal ulcer with Helicobacter pylori infection, which disclosed GIM and chronic gastritis; 3 (n = 25), gastric biopsy specimens from corpus mucosa obtained from patients with autoimmune chronic atrophic gastritis and GIM and without H pylori infection; and 4 (n = 25), gastric biopsy specimens from antral mucosa obtained from patients without H pylori infection, disclosing chronic gastritis without evidence of GIM. The biopsy samples of groups 2, 3, and 4 were obtained during routine upper endoscopy. The study was approved by the Ethics Committee of Hospital Mútua de Terrassa, Terrassa, Spain.

Histologic Assessment

All biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin wax. H&E-stained sections were used to classify gastritis according to the revised Sydney classification and gastric tumors according to the classification of Lauren.

GIM was classified as complete (small intestinal type; CIM) or incomplete (colonic type; IIM) based on the H&E stain. CIM was characterized by the presence of absorptive enterocytes with eosinophilic cytoplasm with a well-developed brush border alternating with well-defined goblet cells. Paneth cells were also frequently present. IIM was characterized by columnar cells without a clear brush border and by the presence of many goblet and mucous cells of varying sizes and shapes. Two pathologists blindly assessed all biopsy specimens on coded slides. Their interobserver agreement was fair (κ coefficient, 0.473). Major problems occurred in the classification of mixed cases. Because only IIM has been suggested to increase the risk of gastric cancer, all mixed cases were considered by consensus as IIM.

H pylori status was assessed by histologic examination and by the urease test in patients represented in groups 2, 3, and 4. A positive histologic examination result required the direct visualization of the organism with routine H&E staining. Both tests were positive in all patients with H pylori infection.

Immunohistochemical Studies

Five new 4-μm-thick sections were cut and used for immunohistochemical studies. Tissue sections from each paraffin block were immunostained with Das-1. The antibody was kindly provided by Kiron M. Das, MD, PhD (Robert Wood Johnson Medical School, Piscataway, NJ). Standard technical procedures were followed. Briefly, after heat-induced antigen retrieval, tissue sections were incubated with primary antibodies for 30 minutes at room temperature. According to Mirza et al,9 the expression of Das-1 was considered positive if a substantial number of cells and more than 1 gland were stained. If only an occasional goblet cell was stained, the sample was considered negative.

A panel of 3 monoclonal antibodies was used to determine the expression of mucins: MUC1 (clone Ma522), MUC2 (clone Ccp58), and MUC5AC (clone CIH2). The pattern of mucin expression was evaluated only in group 1 (away from the tumor) and 2 (n = 75). Seven cases were not evaluated because GIM was not found in the new sections obtained for mucin determination. In addition, the type of GIM was reevaluated in the new sections by 2 blinded pathologists, as described before. This last assessment was used to study mucin expression in relation to the type of GIM. Antibodies were obtained from Novocastra (Newcastle upon Tyne, England). The DAKO EnVision Dual Link (Glostrup, Denmark) was used as a detection system. Diaminobenzidine was used as a chromogen.

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences software, 13.0 release for Windows (SPSS, Chicago, IL). The χ2 or Fisher exact test was used to analyze differences between qualitative variables. The Freeman-Halton extension of the Fisher exact
probability test for a $2 \times 3$ contingency table was used to analyze the differences in the frequency of Das-1 reactivity among the 3 GIM groups. In addition, the McNemar test was used to assess differences between correlated proportions. The level of significance was set at a $P$ value less than .05.

The interobserver agreement in assessing GIM was analyzed by using the $\kappa$ correlation index, with the use of $\kappa$ statistics. The benchmarks suggested by Svanholm et al were accepted. Values less than 0.5 represent fair, values between 0.5 and 0.75 represent good, and values of more than 0.75 represent excellent interobserver agreement. Only values greater than 0.5 were considered good enough for diagnostic reliability.

**Results**

**Patients**

The demographic data for patients are shown in Table 1. There were no statistically significant differences among the 4 groups. In group 1, 31 (62%) patients had intestinal adenocarcinoma, 9 (18%) had diffuse adenocarcinoma, and 10 (20%) mixed adenocarcinoma.

**Das-1 Expression**

Das-1 expression results are summarized in Table 2. In group 1, Das-1 immunostaining was observed in 20 (40%) of 50 cases in cancer areas, but in only 13 (26%) in GIM areas away from the cancer. Das-1 expression was concomitantly observed in tumoral and nontumoral samples in only 7 (14%) of 50 cases. In tumoral tissue, immunostaining was intense in diffuse-type adenocarcinoma and more weak in the intestinal type, where it was localized almost exclusively at the apical pole of neoplastic glands. With respect to the cancer associated metaplastic mucosa, Das-1 was observed in IIM (8 cases) but also in CIM (5 cases) types in goblet and columnar cells. In goblet cells, Das-1 expression was inconstant, with only some cells stained in positive areas. In columnar cells Das-1 positivity was more intense at the apical pole of the cell.

In group 2, Das-1 immunostaining was observed only in CIM glands in 2 (8%) of 25 cases. Das-1 immunoreactivity was absent in all cases in groups 3 and 4.

The expression of Das-1 in GIM from tumor cases away from the cancer area (group 1) was significantly different from that in GIM from nontumor cases (groups 2 and 3): 13 (26%) of 50 vs 2 (8%) of 25 and 0 (0%) of 25 ($P = .004$). There were no significant differences in Das-1 expression between the 2 types of GIM in group 1 (away from the tumor): CIM 5 (22%) of 23 vs IIM 8 (30%) of 27 ($P = .8$; Image 1). There also were no differences in Das-1 expression among the types of gastric adenocarcinoma according to the Lauren classification: intestinal adenocarcinoma, 8 (26%) of 31 cases; diffuse adenocarcinoma, 2 (22%) of 9 cases; and mixed adenocarcinoma, 3 (30%) of 10 cases.

**Expression Pattern of Mucins**

There were no significant differences in the type of mucin expression between groups 1 and 2. Most patients had a mixed mucin pattern consisting of neoexpression of MUC2, underexpression of MUC1, and preserved expression of MUC5AC Image 1. In this sense, there were no significant differences between the subtypes of GIM (based on H&E staining) (Table 3). The mucin pattern previously described in CIM (neoexpression of MUC2 and underexpression of MUC1 and MUC5AC) was observed in 10 cases (1 in group 1 and 9 in group 2), whereas the mucin pattern of IIM (neoexpression of MUC2 and preserved expression of MUC1 and MUC5AC) was seen in only 1 patient in group 1.

There were also no differences in the type of cells expressing mucins. In 56 CIM cases, MUC2 was expressed only in goblet cells in 31 cases (55%) and in goblet and columnar cells in 19 cases (34%), whereas in 6 cases (11%), there was no expression. In 44 IIM cases, it was expressed in 26 (59%) only in the goblet cells and in goblet and columnar cells in 16 (36%), whereas in 2 (5%), there was no expression. MUC5AC expression in CIM was 10 (18%) in goblet cells, 2 (4%) in

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**Table 1**

Demographic Data for Study Groups*

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (SD) Age (y)</th>
<th>Males (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 50)</td>
<td>69.94 (13.2)</td>
<td>48</td>
</tr>
<tr>
<td>2 (n = 25)</td>
<td>53.88 (12.4)</td>
<td>68</td>
</tr>
<tr>
<td>3 (n = 25)</td>
<td>68.2 (14.4)</td>
<td>64</td>
</tr>
<tr>
<td>4 (n = 25)</td>
<td>47.66 (12)</td>
<td>52</td>
</tr>
</tbody>
</table>

* There were no statistically significant differences.

**Table 2**

Results of Monoclonal Antibody Das-1 Expression in Study Groups, Generally and Specific to the Type of GIM*

<table>
<thead>
<tr>
<th>Group</th>
<th>Overall</th>
<th>CIM</th>
<th>IIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 50)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2, GIM (n = 25)</td>
<td>2/25 (8)</td>
<td>2/17 (12)</td>
<td>0/8 (0)</td>
</tr>
<tr>
<td>3, GIM (n = 25)</td>
<td>0/25 (0)</td>
<td>0/16 (0)</td>
<td>0/9 (0)</td>
</tr>
<tr>
<td>4, Gastritis (n = 25)</td>
<td>0/25 (0)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

CIM, complete (small intestinal type) intestinal metaplasia; GIM, gastric intestinal metaplasia; IIM, incomplete (colonic type) intestinal metaplasia.

* Data are given as number/total (percentage).
columnar cells, and 28 (50%) in both types of cells; in 16 (29%) there was no expression. In IIM, it was expressed only in goblet cells in 3 (6%), 4 (10%) only in columnar cells, and 28 (64%) in both; in 9 (20%) there was no expression.

Because Das-1 expression was predominantly seen in GIM in group 1 away from the tumor, the relationship between mucin and Das-1 expression was studied only in such cases. MUC2 and MUC5AC expression was seen in positive

**Table 3**

Expression of Mucins in Subtypes of GIM (Based on H&E Stain) in Groups 1 and 2 (Away From the Tumor) *

<table>
<thead>
<tr>
<th>Mucin</th>
<th>Overall</th>
<th>CIM</th>
<th>IIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUC1</td>
<td>1/44 (2)</td>
<td>0/15 (0)</td>
<td>1/29 (3)</td>
</tr>
<tr>
<td>MUC2</td>
<td>41/44 (93)</td>
<td>13/15 (87)</td>
<td>28/29 (97)</td>
</tr>
<tr>
<td>MUC5AC</td>
<td>29/44 (66)</td>
<td>8/15 (53)</td>
<td>21/29 (72)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mucin</th>
<th>Overall</th>
<th>CIM</th>
<th>IIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUC1</td>
<td>2/24 (8)</td>
<td>2/13 (15)</td>
<td>0/11 (0)</td>
</tr>
<tr>
<td>MUC2</td>
<td>22/24 (92)</td>
<td>12/13 (92)</td>
<td>10/11 (91)</td>
</tr>
<tr>
<td>MUC5AC</td>
<td>23/24 (96)</td>
<td>12/13 (92)</td>
<td>11/11 (100)</td>
</tr>
</tbody>
</table>
Table 4. No specific mucin pattern was associated with Das-1 reactivity ($P < .0005$).

In gastric cancer tissue (group 1), MUC2 and MUC5AC were consistently expressed, without differences within the cancer types. In contrast, MUC1 was underexpressed ($Table 5$).

Discussion

Results of the present study show that Das-1 reactivity was associated with GIM from patients with gastric cancer. The association was much weaker than in the study by Mirza et al, but still statistically significant. However, our results argue against the routine clinical usefulness of this marker because it reacts with only 26% of samples of GIM associated with gastric adenocarcinoma and with 40% of samples of tumor tissue. The reason for the discordant results between the 2 studies is unknown. In agreement with our results, there is a recent study evaluating the usefulness of Das-1 in distinguishing primary gastric adenocarcinoma from metastatic breast adenocarcinoma, in which Das-1 was positive in 43% of gastric adenocarcinomas, a percentage similar to that observed in the present study.

Some possible explanations may be hypothesized to explain the discordant results. First, differences in the type of gastric adenocarcinoma may be at play because GIM is more often associated with the intestinal type. However, this...
mixed adenocarcinoma (n = 10) and diffuse adenocarcinoma (n = 9) were observed. In contrast with this hypothesis, we observed that Das-1 expression in patients without gastric cancer was 70%.

In addition, there was no relationship between the mucin pattern and Das-1 expression. In previous studies, it was suggested that all GIM types showed de novo expression of MUC2, whereas the expression of MUC1 and MUC5AC was decreased in the complete type of IIM and preserved in the incomplete type.

Table 5

<table>
<thead>
<tr>
<th>Expression of Mucins in Tumoral Tissue in Group 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauren Classification</td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>Intestinal adenocarcinoma (n = 31)</td>
</tr>
<tr>
<td>Diffuse adenocarcinoma (n = 9)</td>
</tr>
<tr>
<td>Mixed adenocarcinoma (n = 10)</td>
</tr>
</tbody>
</table>

* P = .31.
† P = .25.

highly subjective. In most previous studies, the possibility of interobserver and intraobserver agreement was not explored. The results of the present study showed a low concordance between the 2 expert pathologists and that GIM classification on different levels from the same specimens yielded significantly different results. Thus, our results reinforce the suggestion that GIM subtyping using standard histologic criteria is a highly subjective procedure, which might explain the discordant results. In this sense, the association of Das-1 with IIM observed by Mirza et al should be viewed with some circumspection.

Immunohistochemical analysis of the mucin pattern in gastric mucosa has been used to differentiate the GIM subtypes, but it is also considered a subjective method. However, there are many published studies using the mucin pattern to identify IIM. Thus, we decided to assess whether it was valuable for recognizing the risk group with GIM associated with gastric cancer and to evaluate the association with Das-1 expression. Results showed that the mucin pattern was not useful for distinguishing between the complete and incomplete types of GIM and, furthermore, for identifying GIM associated with cancer. In addition, there was no relationship between the mucin pattern and Das-1 expression.

In contrast with Mirza et al, we did not find differences in Das-1 expression among the types of gastric adenocarcinoma according to the Lauren classification. Second, differences in the prevalence of *H. pylori* infection in the populations under study may be a factor. The prevalence of the infection in Spain is higher than in the United States. In a nationwide multicenter study in Spain, the prevalence of the infection in patients with gastric cancer was 70%. In fact, Mirza et al described higher Das-1 expression in *H. pylori*+ compared with *H. pylori*− patients (68% vs 25%) in a subgroup of patients from Japan without cancer. In contrast with this hypothesis, we observed that Das-1 expression in patients with GIM without cancer and a positive *H. pylori* finding was only 8% (group 2). In agreement with the present results, there is another study in the literature assessing Das-1 expression in GIM of patients without gastric cancer. *H. pylori* infection was present in 70% of them, but scarce Das-1 immunoreactivity was seen, without differences between positive and negative *H. pylori* cases.

Finally, there are known geographic differences in the incidence of gastric carcinoma among Spain, the United States, and Japan, which suggest that there may be different genetic and environmental factors for gastric carcinogenesis, and Das-1 could be a potential biomarker of gastric cancer in some geographic areas but not in others.

In contrast with Mirza et al, we did not find any association between Das-1 reactivity and the GIM subtypes. Although scarcely documented in the literature, most pathologists agree that the differentiation of GIM subtypes is highly subjective. In most previous studies, the possibility of interobserver and intraobserver agreement was not explored. The results of the present study showed a low concordance between the 2 expert pathologists and that GIM classification on different levels from the same specimens yielded significantly different results. Thus, our results reinforce the suggestion that GIM subtyping using standard histologic criteria is a highly subjective procedure, which might explain the discordant results. In this sense, the association of Das-1 with IIM observed by Mirza et al should be viewed with some circumspection.

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References


