Gastric intestinal metaplasia as detected by a monoclonal antibody is highly associated with gastric adenocarcinoma


Background: Some forms of gastric intestinal metaplasia (GIM) may be precancerous but the cellular phenotype that predisposes to gastric carcinogenesis is not well characterised. Mucin staining, as a means of differentiating GIM, is difficult. A monoclonal antibody, mAb Das-1 (initially called 7E12H12), whose staining is phenotypically specific to colon epithelium, was used to investigate this issue.

Methods: Using mAb Das-1, by a sensitive immunoperoxidase assay, we examined histologically confirmed GIM specimens from two countries, the USA and Japan. A total of 150 patients comprised three groups: group A, GIM (fields away from the cancer area) from patients with gastric carcinoma (n=60); group B, GIM with chronic gastritis (without gastric carcinoma) (n=72); and group C, chronic gastritis without GIM (n=18).

Results: Fifty six of 60 (93%) patients with GIM (both goblet and non-goblet metaplastic cells) from group A reacted intensely with mAb Das-1. Cancer areas from the same 56 patients also reacted. In contrast, 25/72 (35%) samples of GIM from patients in group B reacted with mAb Das-1 (group A v B, p<0.0001). None of the samples from group C reacted with the mAb.

Conclusions: Reactivity of mAb Das-1 is clinically useful to simplify and differentiate the phenotypes of GIM. The colonic phenotype of GIM, as identified by mAb Das-1, is strongly associated with gastric carcinoma.

Materials and Methods
Paraffin embedded tissue blocks were obtained from 150 patients from NJ and Japan.

Group A (n=60)
We used the computer database of the pathology departments to randomly select 60 tissue blocks with a diagnosis of GIM associated with gastric carcinoma. Thirty five patients from Japan (group A1) and 25 from NJ (group A2) were included.

Abbreviations: AB, alcian blue; GIM, gastric intestinal metaplasia; HID, high iron diamine; PAS, periodic acid-Schiff; mAb, monoclonal antibody; H&E, haematoxylin-eosin, NJ, New Jersey.
For each of these 60 patients, paired samples of stomach (surgical specimens) included both cancer areas and histologically proved GIM areas away from the cancer segments.

Group B (n=72)
Biopsy tissue specimens containing GIM without gastric carcinoma were obtained both from Japan (group B1, n=31) and NJ (group B2, n=41).

Group C (n=18)
Eighteen biopsy samples were obtained from Japan with chronic gastritis, without evidence of GIM. The biopsy samples in groups B and C were obtained during routine upper endoscopy procedures for a variety of indications (usually dyspepsia and peptic ulcer disease) other than gastric carcinoma.

Five serial 5 µm sections were obtained from all of the blocks. Haematoxylin–eosin–H&E staining was performed on the first and last section cut from each block and reviewed to ensure the presence of histological abnormalities in both of the sections from the same block. The second to fourth sections were used for immunohistochemical studies. All samples from groups A and B (excluding cancer blocks) had GIM that was observed in both the initial and final H&E sections. One of the sections from each patient was examined with mAb Das-1 using a sensitive immunoperoxidase assay, as described previously. Each experiment also included at least two slides of normal colon and jejunal tissue sections as positive and negative controls, respectively. Reactivity to mAb Das-1 was considered positive if a crisp golden brown staining of cells was present. Two investigators (ZKM and KMD) and a single pathologist (PSA) reviewed each slide together. A substan- tial number of cells, and more than one gland, had to be reactive to mAb Das-1 before a specimen was considered positive. If only an occasional goblet cell was stained, the sample was considered negative. There was agreement among the investigators more than 95% of the time.

Using standard protocols, 76/132 GIM tissue samples (group A, 35; group B, 41) were also stained with AB (pH 2.5)/PAS and AB (pH 2.5)/HID to further subcategorise GIM into various types or “mixed” varieties, based on different colours of mucins displayed by neutral versus sialo or sulphomucins. The remaining tissue samples were not available for mucin staining because of the small size of the tissue in which deeper sections did not have enough mucosa. “Mixed” variety was defined as the presence of both complete and incomplete GIM types in the same tissue section, without a clear predominance of either type. A sample was considered having “predomi- nantly” complete or incomplete if either variety represented more than 75% of the GIM area. Coded slides of mucin staining were interpreted by the single pathologist (PSA). Subsequently, the code was broken and staining of mAb Das-1 and mucin stains were compared to identify the corresponding areas.

**Table 1  Patient demographics**

<table>
<thead>
<tr>
<th>Group*</th>
<th>Mean (SD) age (y)</th>
<th>Males (%)</th>
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<tbody>
<tr>
<td>Group A (n=60)</td>
<td>68.8 (10.0)</td>
<td>60</td>
</tr>
<tr>
<td>Group B (n=72)</td>
<td>64.9 (17.5)</td>
<td>53</td>
</tr>
<tr>
<td>Group C (n=18)</td>
<td>62.8 (15)</td>
<td>72</td>
</tr>
</tbody>
</table>

*Group A included gastric intestinal metaplasia (GIM) tissue away from the cancer areas as well as cancer tissue (surgical specimens) in patients from both Japan (n=35) and New Jersey (n=25). Group B represents GIM tissue (biopsy specimens) from patients without gastric carcinoma (from Japan (n=31) and New Jersey (n=41)). Group C represents gastric mucosal biopsy specimens with chronic gastritis with or without ulcer, but no GIM.

**Figure 1**  Group A represents gastric intestinal metaplasia (GIM) tissue from patients with gastric carcinoma located away from the cancer area, group B represents GIM tissue from patients without gastric carcinoma, and group C represents gastric mucosal biopsies (with no GIM) obtained during routine upper endoscopy (used as controls). (A) Statistical analyses among the total patient groups from Japan and New Jersey (NJ). (B) Differences in each group and between the two countries. A1 versus B1, p<0.0001; A2 versus B2, p<0.01; A1 versus A2, not significant; B1 versus B2, p<0.0001; A1 versus A2, not significant; B1 versus B2, p<0.01.

**RESULTS**

Demographic data of patients in group A (GIM from patients with gastric carcinoma), group B (GIM without associated gastric carcinoma), and group C (chronic gastritis tissue without GIM) are shown in table 1. There were no statistically significant differences among the three groups. Within group A, mean age (SD) for Japanese patients (group A1) was 65.5 (9) years (range 31–92) and mean age of patients from NJ (group A2) was 78.4 (5.9) years (range 69–91) (p<0.002). However, in group B, there were no significant differences in age; mean (SD) age of patients from Japan (group B1) was 65.9 (9) years (range 43–82) and mean age of patients from NJ (group B2) was 78.4 (5.9) years (range 69–91) (p<0.002). In group C, the mean age (SD) for Japanese patients was 62.8 (15) years (range 69–91) (p<0.01). The results of the gastric tissue were negative. In contrast with group A, only 25/72 (35%) GIM samples from patients without gastric carcinoma (group B) reacted with mAb Das-1 (fig 3C) while 63% did not react (fig 3F). None of the samples with...
chronic gastritis without GIM (group C) reacted with the antibody. The difference between the immunoreactivity of mAb Das-1 against GIM in group A versus group B and group B versus C was highly statistically significant (p<0.0001 and p<0.003, respectively) (fig 1A). The cancer area from each of the 56/60 patients from group A, whose GIM tissue samples (away from the cancer area) were positive with mAb Das-1, also reacted with the mAb (fig 4).

Although the reactivity of mAb Das-1 against GIM associated with cancer was similar in patients from Japan (A, B, and C) and in patients from outside Japan (D and E), the reactivity was stronger in group A and B than in group C. The reactivity of mAb Das-1 against GIM associated with cancer was similar in patients from Japan and in patients from outside Japan. The reactivity of mAb Das-1 against GIM associated with cancer was similar in patients from Japan and in patients from outside Japan.
and NJ (A), in patients with GIM without cancer (group B) the reactivity in Japanese patients (subgroup B1) was higher (52%) compared with NJ patients (22%) (subgroup B2). This difference was statistically significant (p<0.01) (fig 1B). The difference between patients in subgroups B1 and B2 may be due to a higher prevalence of Helicobacter pylori infection in Japanese patients. H pylori status was available for each of 31 Japanese patients (group B1) but not for the NJ group (B2). Nineteen of 31 (61%) patients were H pylori positive whereas 12 were negative. Indeed, among these Japanese patients, mAb Das-1 reactivity was higher (13/19; 68%) in the GIM associated with the H pylori positive group compared with the H pylori negative group (3/12; 25%). This difference was highly statistically significant (p<0.001).

Each of the samples of GIM that reacted positively with mAb Das-1 showed intense, mostly cytoplasmic, staining in the goblet cells. In non-goblet metaplastic cells, reactivity was more intense in the basolateral and apical areas, with light cytoplasmic staining. In the cancer tissue, staining with mAb Das-1 was intense, diffuse, and mostly cytoplasmic (fig 4). Portions of the normal gastric mucosa, including the stroma, did not react with mAb Das-1. On reviewing mucin stains (AB/PAS and AB/HID), the majority of the slides showed coexistence of “complete” and “incomplete” GIM phenotypes, with a predominance of either variety, or clearly a “mixed” pattern of staining (table 2). Mapping of selected areas of stained slides showed a consistent correlation between the predominantly “incomplete” type of GIM (type II or III) and mAb Das-1 immunoreactivity (table 2).

Irrespective of group A or group B, 9/10 (90%) of the “incomplete” type of GIM reacted with mAb Das-1 (table 2). However, 12/41 (29%) “complete type” (type I, or small intestinal phenotype) of GIM also reacted with mAb Das-1, suggesting the difficulty in clear separation of incomplete versus complete types of GIM. Nine of these 12 “complete” types that were reactive to mAb Das-1 belonged to group A (GIM associated with cancer) and the remaining three were from group B (GIM without cancer). This difference between groups A and B (9/11 [82%] v 3/30 [10%]) was highly significant (p<0.0001) (table 2). Thus it is clearly evident that mAb Das-1 reactivity is strongly associated with GIM in the presence of gastric cancer, irrespective of “complete” (type 1) or “incomplete” (types II or III) types, with 82% and 100% positivity, respectively (table 2). The cancer areas from each of the same patients (paired samples) also reacted with mAb Das-1.

Eighteen of 35 (51%) GIM samples in group A and 7/41 (17%) in group B were of “mixed” variety, as defined by mucin histochemistry. Of these, 18/18 (100%) samples in group A and 3/7 (43%) in group B were positive with mAb Das-1, further supporting the assertion of the ability of mAb Das-1 to identify the “high risk” phenotype in the “mixed” group.

DISCUSSION

In this study, we have described a monoclonal antibody (mAb Das-1) that showed immunoreactivity in 93% of specimens of GIM associated with gastric carcinoma (group A) but away from the cancer areas. Furthermore, the gastric carcinoma areas from the same 56/60 patients also strongly reacted with mAb Das-1. Immunoreactivity of mAb Das-1 in this group was similar in both Japanese and NJ patients. However, immunoreactivity was significantly (p<0.0001) higher in group A compared with GIM in non-cancer (group B) patients, both from Japan and NJ. Using patients from Japan who were at a lower risk of gastric cancer, the immunoreactivity of mAb Das-1 was similar in group B compared with patients from NJ. The increased immunoreactivity of mAb Das-1 in group A compared with group B indicates that this antibody could be useful in the differential diagnosis of GIM associated with carcinoma.

Table 2: Comparison of monoclonal antibody mAb Das-1 and mucin staining (alcian blue/periodic acid-Schiff and alcian blue/high iron diamine) in gastric intestinal metaplasia (GIM)*

<table>
<thead>
<tr>
<th>GIM</th>
<th>MAb Das-1 reactivity</th>
<th>GIM type on the basis of mucin stain</th>
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<tbody>
<tr>
<td></td>
<td>Complete</td>
<td>Incomplete</td>
</tr>
<tr>
<td>Group A (n=35)</td>
<td>Positive (n=33)</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Negative (n=2)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>11</td>
</tr>
<tr>
<td>Group B (n=41)</td>
<td>Positive (n=9)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Negative (n=32)</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30</td>
</tr>
</tbody>
</table>

*76/132 samples were available for mucin stainings. TGI areas only, away from the cancer segments. 9/11 [82%] versus 3/30 [10%]: p<0.0001.

Figure 4: Haematoxylin-eosin (A) and immunoperoxidase (B) staining of the serial sections from a gastric carcinoma specimen. Intense cytoplasmic staining of the cancer cells with monoclonal antibody mAb Das-1 is clearly evident (original magnification 160×).
higher risk than those from NJ, the data also showed complete lack of reactivity of mAb Das-1 in chronic gastritis in the absence of GIM (group C). These data demonstrate that GIM recognised by mAb Das-1, including both incomplete or colonic-type and complete or small intestinal-type, carries a risk of developing gastric carcinoma.

Gastric adenocarcinoma is the second most common malignancy and cause of cancer related death worldwide. The prognosis of patients with surgically treated early gastric carcinoma, defined as carcinoma confined to the mucosa or lamina propria, is significantly better than those from NJ (group A), Thirty of 35 (86%) patients in group A, were positive for H pylori. Reactivity with mAb Das-1 against this group (A) was significantly higher than in the subgroup B (GIM without carcinoma patients from Japan) (94% v 52%; p<0.01). Similarly, immunoreactivity with mAb Das-1 in GIM from NJ patients, with and without carcinoma (A, v B), was significantly different (92% v 22%, p<0.0001). These data suggest that chronic H pylori infection clearly predisposes to the colonic phenotype of GIM although there may be additional factors for gastric carcinogenesis. However, irrespective of geographical location and aetiological factors, colonic metaplasia is strongly associated with gastric carcinoma. Geographic differences in the incidence and distribution of GIM have also been reported, although the phenotype of GIM in these particular patients is unknown.

In a recent study by Glickman et al, the use of mAb Das-1 was studied in determining the cellular phenotype of Barrett’s oesophagus and GIM from various gastric locations in patients without a history of gastric carcinoma. All cases in their study of a complete-type of GIM from the gastric antrum were negative for mAb Das-1, stressing the fact that there is a lack of incomplete or colonic phenotype in non-cancer patients. Furthermore, in this study from Boston, the frequency of mAb Das-1 reactivity in GIM from the antrum was comparable with our group B patients from NJ (13% v 22%). In our previous reports, mAb Das-1 showed a remarkable sensitivity and specificity in detecting Barrett’s oesophagus, and has helped differentiate Barrett’s oesophagus from GIM and eosinophilic oesophagitis. The 95% reactivity of mAb Das-1 with specialised columnar epithelium in Barrett’s oesophagus by us and by others, has been strongly associated with adenocarcinoma of the oesophagus, can be compared with the 93% reactivity in the GIM from gastric carcinoma patients, suggesting a precancerous stage that is detected by mAb Das-1. This is further supported by the fact that the cancer areas from the same 56/60 (93%) patients with gastric carcinoma also reacted with the antibody. Indeed, staining in the cancer cells was, in general, more intense and diffuse. These data, in addition to earlier reports, strongly suggest that a metastatic process of colonic phenotype is involved in the histogenesis of most of the gastric carcinoma. That mAb Das-1 recognises an epitope associated with precancerous states of epithelial tissue is also supported by its reactivity against chronic cystitis profunda that leads to bladder carcinoma and to adenomatous polyps of the small intestine, particularly associated with familial polyposis and with small intestinal carcinoma. The smaller percentage (approximately 10%) of gastric carcinomas that did not react with mAb Das-1 may have a different cellular phenotype. Indeed, 2/4 non-reactive cancers were histologically of the diffuse-type. In our earlier study, for example, each of the 13 squamous cell carcinomas of the oesophagus did not react with mAb Das-1, and similarly, carcinoma of the prostate did not react with mAb Das-1, whereas carcinoma of the bladder did react.
In conclusion, staining a parallel paraffin section of GIM with mAb Das-1, in addition to the standard H&E and AB/PAS/HID stains, can be useful in differentiating the colonic cellular phenotype of IM that is more highly associated with gastric carcinoma. Its reactivity is clearly evident in GIM as associated with ulcerative colitis: cellular localization of the antigen by using the monoclonal antibody. J Immunol 1987;139:77–84.  

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