Fetal Gastric and Small Intestine Pattern of Intestinal Mucus Antigens in Human Gastric Carcinomas

Jeannette Nardelli, Brigitte London-Rosa, Jacques Bara, and Pierre Burtin

ABSTRACT

The present immunohistological study was performed to investigate the expression of intestinal mucus-associated antigens in different histological types of gastric carcinoma according to the classification of Lauren and the WHO classification. We used the following antigenic markers: M3, present in all the goblet cells of the whole small and large intestine; M3SI, expressed in all the goblet cells of the small intestine, but only in some of them of the large intestine; M3D, mainly produced in the upper small intestine; and M3C, specific for colonic mucus cells.

All these antigens were found to be similarly expressed in both Lauren's intestinal and diffuse types. Nevertheless, M3 and M3C appeared to be more largely produced in carcinomas showing well-differentiated cells (tubulopapillary, mucinous, and signet ring cells according to the WHO classification).

Our results evidenced the occurrence of two main fetal antigenic patterns in gastric carcinomas, one of the gastric type (M3SI produced to a larger extent than M3 and M3C) and the other one of the small intestinal type (M3 and M3SI more largely expressed than M3C). On the basis of their similar antigenic pattern, the histogenesis of carcinomas showing the fetal small intestinal antigenic profile may be associated with intestinal metaplasia. On the other hand, carcinomas with the fetal gastric pattern may originate from undifferentiated stem cells of the gastric mucosa. Thus, such immunohistological studies could lead to a better understanding of the histogenesis of gastric carcinomas.

INTRODUCTION

Although many morphological (10, 21, 28), histochemical (18, 42), and ultrastructural (17, 27) studies have been carried out in the past, the characterization of gastric carcinomas based on differentiation patterns is still far from complete. Based on some particular criteria, several classifications of gastric carcinomas have been proposed. Thus, Lauren divided gastric carcinomas into 2 main groups (21). The first is the intestinal group, including adenocarcinomas with a well-defined glandular formation. In the second group, carcinomas of the diffuse type, tumor cells independently infiltrate the gastric wall, without forming glandular structures. Lauren considered that intestinal features of differentiation could be observed only in the so-called intestinal type. In fact, several later investigations (32) showed the occurrence of intestinal peculiarities in the diffuse type as well. Thus, it appeared that the characterization of gastric carcinomas could not be based on tissular differentiation criteria only. Consequently, other classifications taking into account the tumor growth pattern (27) or both the cellular and tissular differentiation have been proposed more recently.

Using a different approach, i.e., immunohistological technique, we tried in the last past years (1, 31) to further characterize the differentiation pattern of gastric carcinomas. Indeed, several antigens associated with the intestinal mucus (Mi antigens) have been evidenced in our laboratory (see Chart 1). Showing a large (M3, M3SI antigens) or a more restricted (M3D, M3C) localization, these Mi antigens allowed us to define a small and a large intestine antigenic pattern of mucus cells (31) and in addition a fetal gastric antigenic pattern of such cells, which is presented in the present paper. Thus, the immunohistological study of the expression of these Mi antigens in the different types of carcinomas according to the classifications of Lauren (21) and the WHO (40) was undertaken with the following purposes: (a) to characterize the expression pattern of the 4 antigenic specificities associated with the intestinal mucus in the different histological types of gastric carcinomas according to the classifications of Lauren (21) and the WHO (40); (b) to present in evidence an antigen or an antigenic association which could be more closely related to a peculiar histological type; (c) to compare the antigenic profile of each histological type with those of the fetal small intestine and the fetal gastric mucosa; (d) to place our immunohistological results in context with previous morphological, ultrastructural, and epidemiological data toward a better understanding of gastric carcinoma histogenesis.

MATERIALS AND METHODS

Tissues

Adult Tissues. Stomachs, duodenum, and colons were obtained postmortem just after kidney removal for heterotransplantation from trauma subjects maintained in reanimation. From the 16 stomachs collected in this way, samples were taken from cardia, fundus, and pylorus. In 11 cases, mucosa fragments measuring 10 to 15 cm were cut off along the whole lesser and greater curvature and were rolled up in Swiss rolls (24). All these specimens were confirmed as histologically normal.

Fetal Tissues. Twenty-five stomachs were taken from fetuses, which were obtained by abortion or surgery from the Department of Pathology of Cochin Hospital in Paris. The gestational age of the fetuses was determined by measurement of the body length.

Eight fetuses were between 2 and 4 months of gestation, 11 between 4 and 6 months, and 6 between 6 and 9 months. Four whole stomachs from the first group, 8 from the second one, and 5 from the third were coiled in Swiss rolls. From the other specimens, samples were taken from the different parts of the gastrointestinal tract.

Gastric Carcinomas. Two-cm-long tumor fragments (one to 3 from each tumor specimen), never including more than 1 cm of peritumoral gastric mucosa, were taken from fresh gastric resection 1 hr after postmortem.
surgery. All the carcinomas (100 cases) included in our study were histologically confirmed.

All the collected samples were fixed with 95% ethanol.

Antisera

As described previously (31), antisera against antigens associated with the colonic or duodenal mucus were prepared by rabbit immunization with colonic or duodenal high-molecular-weight substances, respectively. Briefly, rabbits received several injections of antigenic material emulsified in complete Freund's adjuvant. Antibodies against blood group specificities and serum components were eliminated by complete absorption with human RBC and polymerized normal human plasma.

For further absorption, pellets of normal gastric, duodenal, and colonic mucosa were prepared by washing insoluble material of each crude extract 3 times with phosphate-buffered saline. Both antisera were thus fully absorbed with normal gastric mucosa extracts (1 volume of pellet/1 volume of antiserum). After such an absorption, the staining of the anti-duodenum mucus serum affected all the goblet cells in the small intestine and only some of them in the large intestine, decreasingly from the proximal to the distal part. This antiserum was then called anti-M3SI.

Further absorption of the same antiserum with colonic extract (1 volume of pellet/1 volume of antiserum) provided evidence for the M3D specificity which was mainly restricted to the upper small intestine.

Likewise, the antiserum specific for the colonic goblet cells, namely, anti-M3C serum, was obtained by additional absorption of the anti-colon mucus serum with duodenal extracts (1 volume of pellet/1 volume of antiserum).

All these absorptions were controlled by indirect immunoperoxidase staining of several sections of normal tissues from the whole gastrointestinal tract.

The preparation of the anti-M3 serum, which reacted with all the goblet cells of the small and large intestine, already had been reported (3).

The staining pattern of the anti-Mi sera in the gastrointestinal tract, as represented in Chart 1, especially the lack of reaction with the gastric mucosa, ruled out any possible contamination of these antisera with antibodies directed against blood group-related antigens, which, like Lea, Leb, x, and y determinants (34), are poorly expressed on RBC but prominent in secretions. Indeed, immunohistological investigations recently carried out by Oriol's group showed that, in addition to the intestinal goblet cells, the surface gastric epithelium was stained with either anti-Lea, anti-Leb, anti-x, or anti-y sera, while gastric glands provided evidence only for the x and y antigens. In contrast, anti-Mi sera did not react with normal gastric mucosa.

Immunoperoxidase

Indirect immunoperoxidase staining of ethanol-fixed and paraffin-embedded sections was performed without any enzyme pretreatment. Endogenous peroxidase activity was blocked by a 30-min preincubation with 5% H2O2. After successive incubations with rabbit antiserum and peroxidase-labeled sheep anti-rabbit IgG (Institut Pasteur Production, France), peroxidase activity was revealed using aminoethylcarbazol as chromophor according to the method of Graham et al. (11). Then, sections were counterstained with 1% hematoxylin and mounted in gelatin-glycerol (Merck, Darmstadt, Germany). Gastric carcinomas were considered as positive for one Mi antigen if at least about 5% of cells in the mucosecretory tumoral area were counted as positive with the corresponding antiserum. The level of expression of one antigen was referred to the extent of the tumoral area stained by the corresponding antiserum. The specificity of the staining was controlled with the antiserum absorbed with the homologous extract.

Histopathology

Tissue specimens were fixed in 95% ethanol and embedded in paraffin according to Sainte-Marie's technique (38). Serial 2-μm-thick sections were cut from tissue blocks with an Autocut (R. Jung, Heidelberg, Germany). They were stained with either hematoxylin-eosin-safran or periodic acid-Schiff or high iron diamine-Aloian blue for sulfomucin and sialomucin visualization (41).

Chart 1. Repartition of the M3, M3SI, M3D, and M3C antigens in the adult and fetal gastrointestinal tract. DUO., duodenum; JEJU., jejunum.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Stomach</th>
<th>Small Intestine</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>M3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M3SI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M3D</td>
<td></td>
<td></td>
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<tr>
<td>M3C</td>
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Antigenic Pattern of the Fetal Gastric Mucosa

Fetal gastric mucosa, especially in the early stages of development, often displays goblet cells among gastric high columnar...
cells of the surface epithelium (42). The antigenic pattern of such goblet cells (see Chart 1) is very similar to that of the fetal small intestine which is characterized by a large production of the M3 and M3SI antigens over M3C and M3D (31). With regard to the typical gastric cells (Table 1) of fetuses older than 4 months, M3SI antigens could be detected in the surface epithelium as well as in the glands of the pylorus, while in some cases, they were present only in the glands of the fundus and cardia. In fetuses younger than 4 months, M3SI antigens were produced in both surface epithelium and glands of either part of the stomach (Fig. 1). In these young fetuses, M3 and M3C antigens could also be observed in some high columnar cells of the surface epithelium, but in much lesser extents than M3SI antigens. M3D staining was never observed in fetal gastric cells.

Antigenic Patterns of Gastric Carcinomas

Classification (Table 2). Among the 100 specimens of gastric carcinomas included in our study, 61 were of the intestinal type, and 39 were of the diffuse type (21).

Forty-one were tubulopapillary, 18 were moderately differentiated, 7 were mucinous, 8 were signet ring cell, and 26 were undifferentiated carcinomas according to the WHO classification (40). Lauren’s intestinal group included all the tubulopapillary and moderately differentiated and 2 of the mucinous carcinomas, while the diffuse type comprised all the other specimens.

Comparison of the Antigenic Pattern between Gastric Carcinomas and the Fetal Gastric and Small Intestine Mucosa (Table 3). Using serial sections of fetal and adult tissues from the different parts of the gastrointestinal tract and taking into account the association and the extent of each Mi antigen, we characterized an antigenic pattern proper to the fetal and the adult gastrointestinal mucosa (see Chart 1). Basing our observations on the same procedure and criteria, we determined for each gastric carcinoma included in our study an antigenic profile, which was compared to the fetal and the adult antigenic pattern.

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Table 1

<table>
<thead>
<tr>
<th>Distribution of the antigenic pattern*</th>
<th>2-4 mo (n = 8)</th>
<th>4-6 mo (n = 11)</th>
<th>6-9 mo (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M3*</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>M3SI</td>
<td>4</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>M3 M3SI M3C</td>
<td>4</td>
<td></td>
<td></td>
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</tbody>
</table>

* Only the antigenic patterns observed in the fetal gastric cells are reported; M3D antigen was never detected in such cells.

Table 2

<table>
<thead>
<tr>
<th>Tumor classification</th>
<th>Lauren</th>
<th>WHO</th>
</tr>
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<tbody>
<tr>
<td>Mi* antigens</td>
<td>I (n = 61)</td>
<td>D (n = 39)</td>
</tr>
<tr>
<td>M3</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>M3SI</td>
<td>50 (86)</td>
<td>30 (75)</td>
</tr>
<tr>
<td>M3D</td>
<td>41 (86)</td>
<td>30 (66)</td>
</tr>
<tr>
<td>M3C</td>
<td>21 (30)</td>
<td>12 (23)</td>
</tr>
<tr>
<td>SRC</td>
<td>38 (62)</td>
<td>21 (53)</td>
</tr>
</tbody>
</table>

* Mi, intestinal mucus; I, intestinal; D, diffuse; TP, tubulopapillary; MD, moderately differentiated; MC, mucinous; SRC, signet ring cell; UND, Undifferentiated.
Table 3: Extent of tumor positivity for M3, M3SI, and M3C antigens in the different types of gastric carcinomas according to the WHO classification. TP, tubulopapillary; MD, moderately differentiated; MC, mucinous; SRC, signet ring cell; UND, undifferentiated. ■, negative; □, <50% of tumor area; ▪, >50% of tumor area.

<table>
<thead>
<tr>
<th>Antigenic profiles</th>
<th>Differentiation pattern</th>
</tr>
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<tbody>
<tr>
<td>M3, M3SI, M3C</td>
<td>Adult stomach</td>
</tr>
<tr>
<td>M3, M3SI, M3C</td>
<td>Adult small intestine</td>
</tr>
<tr>
<td>M3, M3SI, M3C</td>
<td>Fetal stomach</td>
</tr>
<tr>
<td>M3, M3SI, M3C</td>
<td>Fetal small intestine</td>
</tr>
<tr>
<td>M3, M3SI, M3C</td>
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<td>Adult small intestine</td>
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</tr>
<tr>
<td>M3, M3SI, M3C</td>
<td>Fetal small intestine</td>
</tr>
</tbody>
</table>

a The minor antigens are given in parentheses.
b i, intestinal; D, diffuse; TP, tubulopapillary; MD, moderately differentiated; MC, mucinous; SRC, signet ring cell; UND, undifferentiated; Ms, mucosecretory activity characterized by the conventional histochemical methods.

of the gastrointestinal mucosae. Then, it appeared that the fetal gastric and the small intestine patterns were the 2 main antigenic profiles occurring in the different types of gastric carcinomas (Table 3).

Among the other encountered antigenic profiles, some ones could not be associated with any pattern of differentiation (M3, M3M3C), while some others could be reported to a normal adult pattern, either of the gastric type (evidence of a mucosecretory activity without any Mi expression) or of the small intestine type (expression of M3 and M3SI to a larger extent than M3D, absence of M3C).

DISCUSSION

Considering the Laurén’s classification (21), we observed that M3, M3SI, M3D, and M3C were quite equally expressed in both the intestinal and the diffuse type of gastric carcinomas. Thus, our immunohistological results agree completely with previous morphological (25), ultrastructural (27, 32), enzymatic (20, 35), and histochemical (18, 42) investigations which evidenced that, contrary to Laurén’s purposes, intestinal features were not restricted to carcinomas exhibiting a well-defined tissue differentiation. Our 4 intestinal specificities were also recovered in either class of the WHO.

Of several antigens described as associated with the intestinal and/or the colonic mucus, all of them but colonic mucoprotein antigen (8) were recovered in gastric carcinoma (9, 19, 22, 36). Most of the gastric carcinomas, whatever their histological type, expressed more than one antigen and often the 4 specificities. By the use of serial sections, we could observe in the majority of our specimens that, among the antigens present in the same tumor, one of them was expressed to a larger extent than the others. In most cases, the tumor-positive areas corresponding to the major antigen included areas expressing the other minor antigens. This appears to be the case for M3 and M3C antigens, since M3C was very rarely (3 cases) observed in some tumor cells in the absence of the M3 antigen. The connection between M3 and M3SI antigens seemed not so conspicuous. In some well-differentiated carcinomas, the anti-M3 antiserum could sometimes stain only goblet-like cells, while the anti-M3SI antiserum labeled in addition the intervening columnar cells.

Taking into account the association of the antigens and comparing the extent of the expression of each one, we pointed out 2 main antigenic profiles: one corresponding to the fetal stomach; the other corresponding to the fetal small intestine. Both patterns occurred quite equally in the intestinal as well as in the diffuse types of Laurén’s classification. Concerning the classes defined by the WHO, the fetal small intestinal pattern appeared to be more expressed than the fetal gastric pattern in the tubulopapil-
lary (18 of 41 versus 9 of 41) and signet ring cell (6 of 8 versus 2 of 8) carcinomas. No further discrimination could be pointed out in the other histological types of gastric carcinomas.

Such results further improve on those obtained in our previous immunohistological studies of gastric carcinomas. Indeed, when the tumor differentiation was estimated by the mean of a point system as intestinal, gastric, or gastrointestinal, it was first demonstrated that the predominance of the M3 antigen over the gastric M1 and M2 specificities was preferentially associated with the intestinal type of differentiation (1). Then, taking into account M3SI, M3D, and M3C antigens only, we concluded that the intestinal pattern of differentiation may have been related to the fetal small rather than large intestine (31). Concerning the gastric-like differentiation mentioned above (1), we can now specify that the corresponding antigenic pattern should rather be consistent with a fetal gastric pattern.

The occurrence of a fetal pattern of differentiation has been described not only in gastric tumors (5, 12) but also in colon (3, 23) and pancreas (15) carcinomas; it could be thus regarded as a common feature of epithelial tumor cells. However, some tumors exhibit an antigenic profile which does not suggest a fetal pattern of differentiation. Some gastric carcinomas express the M3 antigen but no other antigen associated with the intestinal mucus, and some breast adenocarcinomas produce the M3 antigen as an ectopic feature (4). Moreover, we cannot rule out that some tumor cells differentiate like “normal” cells, according to histological and immunochromoe criteria.

The appearance of the 2 fetal patterns of differentiation, gastric and small intestine-like, in gastric carcinomas should be related to the histogenesis of these tumors. According to the numerous morphological (10, 16, 28), ultrastructural (27), histochemical (13, 18), and epidemiological (6, 43) data already published, there is good evidence that intestinal metaplasia may play a special role in the arising of some gastric carcinomas, not only those of the intestinal type but also some of the diffuse type. Because it has been previously established that intestinal metaplasia adjacent to gastric carcinomas shows a fetal small intestinal antigenic pattern (31), we are tempted to suggest that gastric carcinomas showing that antigenic pattern may probably originate from intestinal metaplasia. Thus, according to our results, the arising of tubuloapapillari and signet ring cell carcinomas might be more closely related to intestinal metaplasia than the other histological types of gastric cancer.

Several authors had already provided evidence for the intestinal pattern of differentiation of signet ring cells (19, 32, 36, 39). According to previously published data (16, 29, 30, 33), we would suggest that the gastric carcinomas which, intestinal or diffuse, show a fetal gastric pattern of differentiation, may arise from gastric undifferentiated foveolar or neck cells. These cells of differentiated gastric cells, when they lose their ability to differentiate as normal gastric cells, could express intestinal features (26, 37) either to a little extent, which according to the present results is to be related to a fetal gastric pattern, or to a large extent, which is associated with the acquisition of a small intestine pattern and with the generation of intestinal metaplasia.

Studies are in progress to observe such fetal features in precancerous conditions or precancerous lesions of the gastric mucosa, as those that have been already reported for the rat (7) and the human colonic mucosa (2, 14). Such investigations may allow us to define some antigenic modifications of the gastric mucosa which could be assigned to a premalignant state and further improve the understanding of gastric carcinoma histogenesis.

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REFERENCES


Fig. 1. Section of gastric mucosa from a 4-month-old fetus stained with anti-M3SI serum. In addition to goblet cells, most gastric cells exhibited a clear staining. Extracellular mucus deposits also reacted strongly with this antiserum. Immunoperoxidase, ×250.

Fig. 2. Section of gastric tumors stained by anti-M3SI. In such a tumor, which was classified in the intestinal and the tubulopapillary groups according to the classification of Lauren and the WHO, respectively, goblet-like cells (arrows) largely expressed the M3SI antigens. In other cells, M3SI antigens were mainly observed in small droplets in the apical cytoplasm. Luminal mucus deposits were also stained (double arrows). Immunoperoxidase, ×250.

Fig. 3. The anti-M3 serum reacted diffusely with the whole cytoplasm of the tumor cells and with the few luminal deposits (arrow) observed in this tumor, which, due to its poor tubular differentiation, was classified in the intestinal and the moderately differentiated groups, according to the classifications of Lauren and the WHO, respectively. Immunoperoxidase, ×250.

Fig. 4. In this carcinoma of the diffuse type, which mainly consisted of typical signet ring cells, most tumor cells displayed a clear M3SI staining. Immunoperoxidase, ×250.
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