A Tumor-associated Antigen in Gastric Cancer Secretions

Emmanuel Deutsch, Charles A. Apffel, Haruki Mori, and John E. Walker

SUMMARY

An antigen, distinct from Gold's carcinoembryonic antigen, was identified in 91% of gastric cancer secretions (GCJ). Reacted with rabbit immune serum against GCJ, this antigen appears as one precipitin line among others on double diffusion in agar gel. When the immune serum has been absorbed with noncancer secretions, it gives a distinctly prevalent line with GCJ both on immunodiffusion and by immunoelectrophoresis. After absorption with normal human plasma, the gastric cancer antigen appears on immunoelectrophoresis as a sharp single line in the \( \beta \) region with a mobility greater than Gold's carcinoembryonic antigen. Gastric cancer antigen has an immunologically reactive carbohydrate moiety and is different from blood group substances. It was not detected in the gastric tumor itself. The corresponding precipitin line disappeared when the immune serum was absorbed with GCJ, thus establishing its pathognomonic significance. Detection of gastric cancer antigen may be crucial for early diagnosis, particularly in younger, still salvageable patients, when there is free HCl on gastric analysis.

INTRODUCTION

The 1st immunochemical analysis of gastric secretions, carried out by Fasel and Scheidegger (5) in 1960, did not include patients with gastric cancer. Subsequent studies (1, 2, 9–13, 18–20, 24, 26, 27) completed the inventory and identification of macromolecular components in normal gastric secretion. In those including gastric cancer (1, 12, 20), either an immune serum against normal gastric secretions was used or the immune serum produced against GCJ was not absorbed. Two reports, one by Bauer et al. (3) and the other by Karitsky and Burtin (14), deal with an autoantigen common to normal and cancerous gastric secretions. The present study is concerned with the search for an antigenic component characteristic of gastric cancer. We have used RAS's to GCJ, produced in cooperation with Dr. S. Hakomori. Specificity was increased by absorption with noncancer juices and with normal plasma.

MATERIALS AND METHODS

Gastric Secretion

Source. One hundred four specimens of gastric secretion were collected at the Carney Hospital, Boston, Mass. Twenty-four were from documented cases of gastric cancer, 22 were from gastric ulcer, 25 were from duodenal ulcer, 16 were from gastritis, 5 were from pernicious anemia, and 12 were from normal subjects or from histologically minimal gastritis. Each pathological case was checked by X-rays, endoscopic examinations, and biopsy of the gastric mucosa or laparotomy. Histological criteria for gastritis were those of Deutsch and Christian (4).

Collection. After placing a nasogastric tube and discarding the fasting specimen, we injected 50 mg Histalog (Eli Lilly & Co., Indianapolis, Ind.) i.m. for secretory stimulation. Gastric secretion was then continuously aspirated for 90 min. Pepsin was inactivated intragastrically by adjusting the pH to 7 at 10-min intervals with NaHCO\(_3\) solution (5 g/100 ml). Patients were cautioned to eject saliva in order to minimize its admixture. Specimens with bile or blood were discarded.

Processing. Samples were withheld to determine pH, free and total acidity, blood group substances, etc. The rest was centrifuged at 1800 \( \times \) g for 10 min. Supernatants were dialyzed against tap water for 48 hr at 6–8° and then lyophilized.

For initial investigations, all the juices were lyophilized. For clinical purposes, we now aspirate for a few minutes only and do not lyophilize.

Antisera

Initially, the lyophilized secretions from 2 patients with proven gastric adenocarcinomas (without stenosis) were used as antisera. In later studies, these were replaced by a pool of 6 lyophilized GCJ's. Ten mg lyophilate were thoroughly mixed with 2 ml 0.9% NaCl solution and emulsified with an equal volume of complete Freund's adjuvant. The mixture was injected into each foot pad and both thighs of a mature rabbit 3 times at 10-day intervals. The sensitized animals were bled, and the immune sera were prepared 10 days after the last injection.3

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Absorption

Antiserum was absorbed with pooled secretions from gastric ulcer, duodenal ulcer, and gastritis. Three mg of each lyophilate, i.e., 9 mg total, were dissolved in 0.3 ml 0.9% NaCl solution. The optimal ratio was 1 volume of absorbing solution to 20 volumes of antiserum. In a 2nd step, anti-GCJ serum was absorbed with pooled NHP in a volume ratio of 9:1. Absorptions were performed at 37° for 30 min and then at 4° for 3 days before final centrifugation of the immune precipitate (21). Finally, anti-GCJ serum was absorbed with GCJ (pool of 6) in order to investigate whether the antiserum would lose its reactivity after absorption with the cancer material.

Immunodiffusion

Secretions from gastric cancer patients as well as from noncancer patients, coded and randomized, were assayed by Ouchterlony's double diffusion test (16). The criterion of positivity was the appearance of a particular precipitin line not fusing with other lines brought about by secretions from noncancer patients if the antiserum was not absorbed. The critical line was dominant if the antiserum had been absorbed with noncancer secretions and single if absorption had been performed with NHP.

For further characterization, anti-GCJ immune serum absorbed with NHP was tested simultaneously against a GCJ known to give the characteristic precipitin line and blood group substances extracted from GCJ. The extraction was performed according to the method of Morgan and King (15). The precipitate containing the blood group activity was dissolved in 0.9% NaCl solution (5 mg/ml).

In special tests, GCJ was reacted simultaneously with the NHP-absorbed anti-GCJ, with Gold's anti-CEA (courtesy of Dr. W. Nugent, Lahey Clinic), and with an antiserum against the "piece" of secretory immunoglobulin A (25), provided by Dr. D. Rowe, WHO, Geneva, Switzerland.

Immunoelectrophoresis

After zone electrophoresis, samples were developed in immunoelectrophoresis against immune sera following the method of Scheidegger (22).

GCJ was also developed simultaneously against anti-GCJ absorbed with NHP and against Gold's anti-CEA serum (Fig. 1).

Periodate Oxidation

One-tenth ml of the GCJ lyophilate solution was treated with 33 mm sodium metaperiodate in the dark at 5° and pH 4.5. The solution was dialyzed at 4° with distilled water for 24 hr and was finally examined by immunoelectrophoresis.

Extraction of GCA from Gastric Cancer Tissue

Minced tumor tissue from a gastric adenocarcinoma was dispersed in a VirTis Model 45 homogenizer at lowest speed for 1 min. The cell dispersate was passed through 2 and then 4 layers of gauze. The cells were spun out at 250 X g for 5 min and washed twice in Krebs' balanced salt solution. One aliquot was subsequently homogenized. The homogenate was centrifuged at 850 X g for 15 min. Another aliquot was gently stirred for 20 min in 0.07 M NaCl + 0.07 M sodium citrate with 0.005 mM disodium EDTA. Finally, the cells were spun out at 300 X g for 10 min.

The saline extract from the homogenate and the supernatant after treatment of whole cells with complexing agents were assayed by immunoelectrophoresis with anti-GCJ serum absorbed with NHP.

RESULTS

Antibodies were detected 20 days after the 1st sensitizing injection of GCJ.

Immunodiffusion. When tested against nonabsorbed antiserum, GCJ gave strong precipitin lines fusing with each other in typical identity patterns (Fig. 24). These lines did not fuse but spurred with much weaker ones brought about by secretions from noncancer patients. Results were essentially the same with antiserum to Case 1 and antiserum to Case 2. The reaction increased in specificity upon absorption with noncancer secretions. Precipitin lines produced by noncancer secretions were reduced in number and weaker in appearance, while those produced by GCJ were unimpaired. When the antiserum was absorbed with human plasma, nonspecific lines disappeared completely, leaving 1 single line characteristic of GCJ. This line corresponds to our criterion of positivity and, as we believe, to a tumor-associated GCA. Accuracy with this technical improvement increased to 93%. This figure represents the ratio 97:104, i.e., \( \frac{|t - (n + \bar{p})|}{t} \), where \( t \) is total of tests, \( n \) is number of false negatives, and \( \bar{p} \) is number of false positives (Table 1). Five false positives corresponded to 3 gastric ulcers, 1 duodenal ulcer, and 1 pernicious anemia. Detection of GCA with this antiserum made it possible to diagnose 1 case of gastric carcinoma (limitis plastica) in a 43-year-old man despite the fact that chemistry (pH of juice was 2), X-rays, and endoscopy were indicative of ulcer. While 4 mucosal biopsies had been negative, the diagnosis was

<table>
<thead>
<tr>
<th>Nature of antiserum</th>
<th>Absorbed with NHP</th>
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<tr>
<td>with noncancer secrections</td>
<td>with noncancer secrections</td>
</tr>
<tr>
<td>with NHP</td>
<td>with NHP</td>
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</tbody>
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- Antigen: Gastric secretion (lyophilate) against anti-GCJ serum

Table 1

Positive reactions by immunodiffusion of gastric secretion (lyophilate) against anti-GCJ serum

<table>
<thead>
<tr>
<th>Source of secretion</th>
<th>No. of specimens</th>
<th>Nonabsorbed</th>
<th>Absorbed with noncancer secretions</th>
<th>Absorbed with NHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric cancer</td>
<td>24</td>
<td>20</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>Pernicious anemia</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>22</td>
<td>9</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>25</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Gastritis</td>
<td>16</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>12</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>
confirmed after laparotomy by a transmural biopsy. Eighteen months later, it became clinically and radiologically evident.

The NHP-absorbed antiserum gave no line with the extracted blood group substances, and the GCA line resulting from interaction with GCJ was unimpaired after the serum was absorbed with erythrocytes of the various ABO blood groups.

Interestingly, 2 out of 4 GCJ's produced a line, different from that of GCA, with immune serum against the "piece" of secretory IgA (25) while noncancer secretions did not. One GCJ, reacted simultaneously with both our absorbed antiserum and Gold's anti-CEA serum, gave a distinct line with our antiserum only.

Immunoelectrophoresis. Reacted with pepsin-inactivated GCJ, the absorbed immune serum gave 7 lines. With normal human serum, instead of GCJ, 6 lines were brought about, seemingly identical with corresponding lines produced by GCJ. However, the 2nd line after the cathode (as it appears on immunoelectrophoregrams with GCJ) was absent. By absorption with NHP, the immune serum against GCJ became monovalent, giving 1 single line in the β region upon reaction with GCJ (Figs. 1A and 3A) but not with noncancer juices. This line represents the tumor-associated antigen, i.e., the GCA herewith described. When the monospecific antiserum was finally absorbed with GCJ, the precipitin line was no longer visible (Fig. 3).

When one of the GCJ's was developed simultaneously against our monovalent anti-GCJ serum and against Gold's anti-CEA (6—8), a single line was obtained with both reagents. However, the line corresponding to our GCA was stronger and sharper, and GCA did migrate faster than CEA (Fig. 1).

Glycoprotein Nature of GCA. Whereas untreated gastric cancer secretion gave 7 precipitin lines, the same treated with metaperiodate produced no lines except the most anionic (albumin fraction). Antigen fractions corresponding to the other 6 lines are, therefore, considered to have immunologically active carbohydrate chains. This includes the line corresponding to GCA.

Localization of GCA. With NHP-absorbed antiserum reagent, the GCA could not be detected in saline extracts of homogenized gastric adenocarcinoma or in the material released from the surface of the tumor cells by complexing agents in isotonic solutions.

DISCUSSION

We emphasize the absolute necessity of the intragastric inactivation of pepsin in order to avoid loss of antigenicity resulting from proteolysis under acidic conditions.

We have detected the presence in gastric secretion of an antigen associated with cancer of the stomach. Apparently, this antigen pertains to GCJ only, is not shared with normal human serum, and represents 1 component of GCJ, which does not originate from the plasma. The fact that it disappears after absorption with the cancer material demonstrates its diagnostic significance.

Our data indicate that GCA is different from the blood group substances contained in gastric secretion and different from the CEA. It may be objected that the difference in mo-bility between GCA and CEA (6—8) observed by us in immunoelectrophoresis is not conclusive although the same antigen was reacted with both antisera. Two other facts, however, strengthen our assumption: (a) one particular GCJ gave a line with our NHP-absorbed antiserum while not interacting with Gold's reagent; (b) GCA could not be extracted from a gastric carcinoma while CEA is extractable. We have also established that GCA is not identical with the "piece" of Tomasi's secretory IgA (24). The observation that some GCJ did react with the anti-"piece" serum while secretions of noncancer patients did not may lead to a new diagnostic criterion. Normally, cells of the gastric mucosa conjugate the "piece" with 2 monomers of serum IgA to produce "secretory IgA." It is indeed conceivable that gastric cancer cells are less capable of this performance because of a functional dedifferentiation parallel to morphological anaplasia. If any relation with Karitsky and Burtin's autoantigens (14) can be ruled out, the identity with 1 or 2 glycoproteins, found by Arakawa (2) to appear in gastric secretions after carcinogenesis, is a distinct possibility.

The incidence of detectable GCA depends on the means of absorbing the antiserum. Absorption of the antiserum with gastric secretion from normal individuals and patients with nonmalignant gastric ailments gives a slightly lower incidence of detectable GCA suggesting quantitative differences in antigen level between GCJ and noncancer gastric secretions. Use of plasma for absorption appears to render the system specific and gives highest incidence of antigen detection. Possibly, absorption of the anti-GCJ serum with both noncancer gastric secretion and plasma will prove to be the means of choice. As of now, we have achieved an overall accuracy of 93% for this clinical trial if we subtract 2 false negatives and 5 false positives (see last column of Table 1) from the total of 104 tests. Our findings, supported by observations of Patterson et al. (17), contribute a means of detecting a tumor-associated antigen before achlorhydria occurs. This applies particularly to "mucosal carcinoma" (23) which has a salvageability rate of 70 to 80% as compared with 5 to 12% for gastric cancer, including all varieties. The latter figure should by the same token be significantly improved.

ACKNOWLEDGMENTS

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Tumor-associated Gastric Antigen


Fig. 1. Groove A, RAS against GCJ absorbed with NHP; well, GCJ (pool of 4). Groove B, RAS against CEA (Gold’s test serum). Notice that each RAS gives a distinct line. The one produced by A corresponds to the GCA, which migrates faster.
Fig. 2. Ouchterlony's immunodiffusion of various gastric juices with antiserum to GCJ. A. Central well, antiserum to GCJ absorbed with noncancer secretions. 25 and 26, GCJ. 27, 28, and 30, gastric and duodenal ulcers. B. Central well, antiserum to GCJ absorbed with NHP. 1 and 2, GCJ. 3 to 6, noncancer secretions.

Fig. 3. A. Well GCJ (pool of 6); groove, RAS against GCJ (pool of 6) absorbed with NHP. B. Well, same as in Well of A; groove, RAS of Groove A absorbed with GCJ (pool of 6). Notice disappearance of GCA precipitin line after absorption with the cancer material.
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