Localization of Human GW-39 Tumors in Hamsters by Radiolabeled Heterospecific Antibody to Carcinoembryonic Antigen

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SUMMARY

The in vivo localization of radiolabeled heterospecific antibody to carcinoembryonic antigen (CEA) was investigated in a human colonic tumor-hamster host model. With the paired-labeled antibody technique, a significant and specific accumulation of goat anti-CEA immunoglobulin G (IgG) was observed in small tumors (<5.0 g) 1 day after injection. The level of anti-CEA IgG in the tumor subsequently decreased but at a slower rate than that for normal IgG in the tumor and both normal and anti-CEA IgG in the reference tissues. The tumor/blood ratio for anti-CEA IgG was approximately four times greater than that for normal IgG 8 days after injection. Absorption of labeled anti-CEA IgG with CEA reduced markedly its preferential accumulation in tumors. Since larger tumors (>5.0 g) failed to show preferential uptake of anti-CEA IgG, it is suggested that circulating antigen and/or hamster antibody against CEA interfere with the accumulation of localizing antibody in larger tumors.

INTRODUCTION

The CEA1 was first described as a specific constituent of human adenocarcinomas arising from endodermally derived gastrointestinal tissues (8). Krupey et al. (15) have isolated and identified CEA as a glycoprotein intimately associated with the tumor cell surface (9). The presence of CEA in the serum of patients with colorectal tumors (33) has led to the development of assay procedures that can detect circulating CEA in patients with other cancers and certain nonneoplastic diseases (16, 19, 22). Although CEA has been demonstrated recently in normal lung, colon, and plasma (1, 17, 20), its concentration in these locations and in the blood of cancer patients is minute relative to the amount extractable from certain tumors.

The presence of CEA in the tumor cell surface suggests that radiolabeled anti-CEA antibodies could be used for in vivo localization of tumors producing this antigen. Earlier studies of tumor localization with antifibrin antibodies yielded limited success in both humans and experimental animals (4, 29–31). More recent investigations with antibodies directed against constituents produced by the tumor have also given equivocal results (26, 27). However, Izzo et al. (14) reported significant tumor localization with antibodies against transplantation antigens of chemically induced rat tumors. We now present evidence supporting the feasibility of localizing CEA-producing human colonic carcinomas in an experimental model.

MATERIALS AND METHODS

One goat, Ro 183, was immunized against purified CEA (E. S. Newman, H. J. Hager, and H. J. Hansen, manuscript in preparation) and served as a source of antiserum for the results described here. Normal goat IgG was obtained commercially (Miles Laboratories, Kankakee, Ill.) from a single lot (No. 15).

The IgG fraction of goat anti-CEA antiserum was prepared by column chromatography on Sephadex G-200 (Pharmacia, Uppsala, Sweden) and DEAE-cellulose (Whatman DE52, H. Reeve Angel, Inc., Clifton, N. J.) (2). The whole IgG fraction of the goat antiserum was designated anti-CEA IgG. The purity of the goat IgG was checked by immunoelectrophoresis and immunodiffusion. Both the anti-CEA and normal IgG were used without prior absorption.

IgG was radioiodinated with either 125I or 131I (Amersham/Searle, Arlington Heights, Ill.) by the procedure of Greenwood et al. (12), as modified by McConahey and Dixon (21). Radioactive IgG was separated from unbound radioactivity by filtration over Sephadex G-100 and was collected in 10% normal hamster serum. Each radiolabeled preparation was shown to be free of aggregate by filtration over Sephadex G-200. The anti-CEA IgG was monitored for immunoreactivity by the radioimmunoassay of Hansen et al. (13), prior to labeling. After labeling, immunoreactivity was checked by incubating the labeled IgG, at equivalence, with purified CEA, and the amount of complexes formed was quantitated by Sephadex G-200 chromatography. The specific activity of the labeled IgG preparations was 4 to 6 μCi/μg.

Male Syrian hamsters (LVG/LAK), weighing 50 to 60 g, were heterografted i.m. with a human signet-ring cell carcinoma of the colon, GW-39 (11). The presence of the CEA in GW-39 has been reported recently (10). At the time of transplantation, I s.c. injection (2.5 mg) of cortisol acetate (Merck, Sharp, and Dohme, Inc., West Point, Pa.) was given. The paired-labeled antibody technique developed...
oped by Pressman et al. (23) was used to evaluate specific uptake of anti-CEA IgG. Depending upon the experiment, tumor-bearing hamsters were given an intracardial injection of the radiolabeled anti-CEA and normal IgG mixture 1 to 4 weeks after implantation of tumor. In most experiments, the anti-CEA and normal IgG were labeled with $^{131}I$ and $^{125}I$, respectively. The radioactivities were mixed 1/1 and sterilize filtered; 10 to 12 µCi of each were given in a final volume of 0.2 to 0.3 ml. Animals were given Lugol's solution in their drinking water to block thyroid uptake. At timed intervals, each animal was exsanguinated by cardiac puncture, various organs (liver, spleen, kidney, lung, stomach, muscle, tumor) were removed, and radioactivity was determined in each and in 1 ml of blood using a Packard Model 3375 $\gamma$ scintillation counter. Using appropriately diluted injection mixture standards, the percentage of injected dose found per g of tissue was calculated for each radioisotope. In addition, a localization ratio was derived using the formula (32) (anti-CEA IgG/normal IgG recovered in tissue)/(anti-CEA IgG/normal IgG injected).

The level of significance between tumors and reference tissues was calculated by Student's $t$ test.

RESULTS

Chart 1 shows the tumor localization ratio at different intervals after injection of an anti-CEA and normal IgG mixture. Values greater than 1.0 demonstrate preferential accumulation of anti-CEA IgG over the normal IgG. At all times, the ratio obtained in the tumors was significantly greater ($p < 0.001$) than the reference tissue ratios which equaled approximately 1.0. The percentage of injected dose per g of tissue on Day 4 of this experiment is shown in Table 1, which also compares the results obtained when the labels on the respective $\gamma$-globulins were reversed. In both, the anti-CEA IgG concentration in the tumor was significantly greater ($p < 0.005$) than its level in the reference tissues. Also, the $^{131}I$ activity in the reference tissues is higher than the level of $^{125}I$ activity, regardless of the $\gamma$-globulin source. This suggests that the metabolism of IgG in this system is altered somewhat by either the nature of the label or the slight variation in radiolabeling conditions that are used for the 2 different nuclides.

The increase in the tumor localization ratio with time (Chart 1) indicates that either the anti-CEA IgG concentration increases in the tumor or the normal IgG disappears from the tumor at a faster rate than the former. After Day 1, both the concentration of anti-CEA and normal IgG in the tumor decrease with time (Chart 2). However, the biological half-life of anti-CEA IgG in the tumor was nearly twice as long as that of the normal IgG. During this same time interval, the clearance of normal IgG from the circulation was not significantly different from that for anti-CEA IgG (Chart 2).

A significant increase in the tumor localization ratio did not appear between Day 6 and 8 of the 1st experiment. However, 3 tumors from Day 8 had a mean weight of 3.24 g and a mean ratio of 2.92, whereas the remaining 3 tumors had a mean weight and ratio of 1.22 and 5.37, respectively. This suggests that tumor size may have a marked influence on the preferential accumulation of anti-CEA IgG. In a separate experiment, the localization ratio in tumors of different sizes was compared on day 4 (Table 2). A decrease in the tumor localization ratio paralleled increasing tumor size, so that in the largest tumor group this ratio was not different from that in the reference tissues with the exception of blood ($p < 0.001$) and liver ($p < 0.05$). A comparison of the level of anti-CEA and normal IgG showed no significant change in the accumulation of normal IgG with increasing tumor size (Table 2). By contrast, there was a marked drop in the level of anti-CEA IgG in the larger tumors. This suggests that the larger tumors have less antigen exposed or available to antibody or that there is a corresponding decrease in antigen synthesis with tumor growth. However, the low blood localization ratio ($0.78 \pm 0.03$) in animals with large tumors at Day 4 suggests that the anti-CEA IgG is cleared from the circulation at a faster rate than is the normal IgG. Since hamsters bearing i.m. GW-39 tumors in this size range circulate CEA (25), it is possible that the injected anti-CEA IgG is cleared rapidly as a CEA-anti-CEA IgG complex before it reaches the tumor site.

In order to confirm that the preferential accumulation of anti-CEA IgG in GW-39 was due to antibody specificity the following experiment was done.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Anti-CEA IgG-$^{125}I$</th>
<th>Normal IgG-$^{131}I$</th>
<th>Anti-CEA IgG-$^{131}I$</th>
<th>Normal IgG-$^{125}I$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>2.86 ± 0.34*</td>
<td>1.14 ± 0.12</td>
<td>3.99 ± 0.68</td>
<td>2.01 ± 0.36</td>
</tr>
<tr>
<td>Liver</td>
<td>0.42 ± 0.04</td>
<td>0.33 ± 0.03</td>
<td>0.51 ± 0.06</td>
<td>0.55 ± 0.06</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.21 ± 0.03</td>
<td>0.17 ± 0.03</td>
<td>0.43 ± 0.07</td>
<td>0.5 ± 0.09</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.53 ± 0.04</td>
<td>0.42 ± 0.03</td>
<td>0.65 ± 0.1</td>
<td>0.76 ± 0.12</td>
</tr>
<tr>
<td>Lung</td>
<td>0.74 ± 0.09</td>
<td>0.57 ± 0.06</td>
<td>1.36 ± 0.18</td>
<td>1.51 ± 0.19</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.63 ± 0.08</td>
<td>0.52 ± 0.07</td>
<td>0.88 ± 0.15</td>
<td>0.98 ± 0.18</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.21 ± 0.08</td>
<td>0.92 ± 0.06</td>
<td>0.75 ± 0.2</td>
<td>0.84 ± 0.22</td>
</tr>
</tbody>
</table>

* No. of animals.
* Mean tumor weight ± S.E. (g).
* Mean ± S.E.
Tumor Localization with Anti-CEA Antibody

Chart 1. Localization ratio obtained in tumors following injection of anti-CEA and normal IgG mixture.

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Tumor wt (g)</th>
<th>Localization ratio</th>
<th>% injected dose/g of tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Anti-CEA IgG</td>
</tr>
<tr>
<td>A</td>
<td>7</td>
<td>0.45 ± 0.07*</td>
<td>2.26 ± 0.12</td>
<td>4.34 ± 0.75</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>3.25 ± 0.48</td>
<td>1.79 ± 0.19</td>
<td>1.68 ± 0.15</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>8.02 ± 0.98</td>
<td>1.00 ± 0.03</td>
<td>1.31 ± 0.05</td>
</tr>
</tbody>
</table>

* Comparison of Group A to B and C.

** Mean ± S.E.

NS, not significant.

Anti-CEA IgG with CEA causes a significant decrease (p < 0.001) in the uptake of this labeled preparation by GW-39 tumors. However, the free IgG that remains after absorption of the anti-CEA IgG still retained some ability to accumulate to a greater extent than the normal IgG in these tumors. There are several alternatives that could explain the elevation of free IgG in the tumor as compared to normal IgG. As mentioned previously and as depicted in Table 1, the γ-globulin bearing the 125I label concentrates in the reference tissues to a higher degree regardless of the IgG source. Furthermore, the increased accumulation of anti-CEA IgG over normal IgG in the reference tissues (Table 3) might reflect differences in metabolic processing of 2 different IgG preparations, such as either variation in animal source or differences in isolation procedures. It is also possible that the goat antiserum to CEA possessed a higher titer of heterophilic antibodies to hamster antigens than the goat serum that served as a source of normal IgG. Hamster tissue stroma in the human GW-39 tumor would then account for the elevation of free IgG in the tumor. Finally, heterogeneity in the antigenic determinants on CEA from...
Table 3

Distribution of unfractionated $^{131}$I-labeled anti-CEA IgG and $^{131}$I-labeled free IgG in tissues 2 days postinjection

The labeled anti-CEA IgG was complexed with CEA and the labeled free IgG was obtained after Sephadex G-200 chromatography.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Unfractionated Anti-CEA IgG-1$^{131}$I</th>
<th>Normal IgG-1$^{131}$I</th>
<th>Free IgG-1$^{131}$I</th>
<th>Normal IgG-1$^{131}$I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>4.75 ± 0.2b</td>
<td>2.55 ± 0.13</td>
<td>3.06 ± 0.24</td>
<td>2.25 ± 0.18</td>
</tr>
<tr>
<td>Liver</td>
<td>0.76 ± 0.02</td>
<td>0.63 ± 0.01</td>
<td>0.71 ± 0.04</td>
<td>0.61 ± 0.03</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.1 ± 0.13</td>
<td>0.88 ± 0.12</td>
<td>0.55 ± 0.07</td>
<td>0.43 ± 0.05</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.31 ± 0.08</td>
<td>1.03 ± 0.07</td>
<td>1.33 ± 0.07</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>Lung</td>
<td>1.75 ± 0.12</td>
<td>1.37 ± 0.1</td>
<td>1.95 ± 0.16</td>
<td>1.5 ± 0.13</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.96 ± 0.09</td>
<td>0.85 ± 0.07</td>
<td>1.11 ± 0.11</td>
<td>0.99 ± 0.09</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
<td>1.87 ± 0.19</td>
<td>1.14 ± 0.15</td>
</tr>
</tbody>
</table>

* No. of animals.
* Mean tumor weight ± S.E. (g).
* Mean ± S.E.

different individuals may explain the failure of a single source of purified CEA to absorb completely the anti-CEA IgG.

As expected, the tumor/blood ratio of the anti-CEA IgG was significantly higher ($p < 0.005$) than that for the normal IgG (Chart 3). Although the tumor/blood ratio of the normal IgG did not change with time, it did show a marked increase for anti-CEA IgG. The data suggest that these tumors can be clearly delineated by photoscanning techniques as early as Day 1, at which time the tumor/blood ratio of the anti-CEA IgG is higher than the corresponding ratio in the reference tissues. At later stages, differentiation between tumor and background activity should increase markedly as the radioactivity is cleared from the other tissues. This relationship has been shown to be satisfactory for localizing by photoscanning (D. M. Goldenberg, D. Preston, F. J. Primus, and H. J. Hansen, submitted for publication) GW-39 tumors complexed with radiolabeled anti-CEA IgG.

**DISCUSSION**

Reif (27) has speculated that the radiolabeled antibody technique may localize CEA-producing intestinal tumors and establish the extent of micrometastatic involvement. The studies reported here demonstrate the high degree of preferential accretion of anti-CEA IgG in the CEA-producing GW-39 tumor. The specific uptake of antibody is largely dependent upon tumor size, being totally suppressed in tumors larger than 5 g. With tumors weighing 5 g or more, CEA ($< 20$ ng/ml) has been observed to be present in the blood of the hamster (25). As previously reported for a mouse myeloma system (27), the presence of circulating antigen in the GW-39 model may explain the failure of antibody to localize in large tumors. Although serum CEA titers were not performed on animals used for tumor localization, other preliminary evidence (unpublished data) indicates that the amount of injected specific antibody was 5 to 10 times greater than the total quantity of circulating CEA. However, if the release of the antigen exceeds the rate at which the antibody accumulates in the tumor, then most of the antibody would fail to reach the tumor due to the rapid elimination of antigen/antibody complexes from the circulation. Since CEA is found circulating in cancer patients, its presence could perhaps abrogate the localizing potential of labeled antibody. Hence, further studies with the GW-39 model are needed in order to evaluate what influence circulating CEA has on tumor localization before clinical use of labeled monospecific antibodies can be pursued.

Although the presence of autoantibodies against CEA in human cancer patients is a subject of much debate (3, 18), Gold (7) and Gold et al. (6) have shown with a variety of methods the existence of an autoantibody against an antigenic site on the CEA molecule. This antibody is not de-
tectable in patients who have extensive metastatic involvement, and it was therefore suggested that the tumor in these cases acted as an antibody “sink” (5). The presence of GW-39 also induces the formation of an IgM anti-CEA antibody in the hamster (25). Although the GW-39 model is a xenogeneic system, this antibody in the hamster attains maximum titer when the tumor is approximately 5 g (25). Thus, it is possible that the deposition of hamster anti-CEA antibody in large tumors masks antigenic sites on the tumor cell surface and prevents their recognition by labeled, localizing antibodies.

In agreement with work performed in dogs and rabbits by Shuster et al. (28), we have found that the clearance of radiolabeled CEA from the circulation of normal hamsters is mediated by the liver (24). However, the elimination of labeled CEA is retarded in tumor-bearing hamsters (24). Since our initial findings showed that hamster antibody titer exceeds circulating CEA concentration (25), it seems likely that this antibody interferes with CEA clearance from tumor-bearing hamsters. It would appear of significance to known whether circulating CEA in cancer patients is also bound to antibody and whether the latter reacts with or masks the determinant(s) responsible for localizing antibody activity. If this were to happen, then presumably the presence of circulating CEA would not inhibit the ability of localizing antibody to reach the tumor. If, however, the affinity of the localizing antibody IgG for CEA favors the displacement of autoantibody from circulating antigen, it is conceivable that a change in antibody class on CEA would lead to rapid elimination of localizing antibody/CEA complexes.

These initial investigations of tumor localization in the GW-39 colonic cancer model not only show the potential usefulness of this approach in humans but also demonstrate the problems that one might anticipate in clinical use. Recent success in applying this procedure to tumor localization by photoscanning (D. M. Goldenberg, D. Preston, F. J. Primus, and H. J. Hansen, submitted for publication), however, strengthens our belief that this approach might indeed have clinical applicability. There are several other related aspects that could also be explored with the GW-39 model. The most significant of these is whether or not the injection of larger quantities of anti-CEA antibodies could induce tumor enhancement or rejection and whether they could serve as a vehicle to carry more selectively anticancer agents into the tumor.

REFERENCES

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