Use of a Passive Hemagglutination Inhibition Test for Monitoring Levels of Serum Carcinoembryonic Antigen Following Surgical Therapy

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ABSTRACT

A technique of passive hemagglutination inhibition (PHI) has been used to monitor levels of carcinoembryonic antigen (CEA) in human sera following surgical therapy. CEA was coupled to human type O-negative erythrocytes in the presence of bis-diazotized benzidine. Pre-operative and post-operative sera from 11 patients with primary adenocarcinomas of the gastrointestinal tract and from one patient with ulcerative colitis were then tested for their capacity to inhibit the agglutination of the sensitized cells in the presence of a predetermined amount of goat anti-CEA serum. Positive sera were defined as those which inhibited agglutination at dilutions of > 1:8.

The pre-operative sera from 11 of the 12 patients inhibited agglutination at dilutions of 1:16 or greater. The one negative serum was from a patient with primary adenocarcinoma of the colon in the stage of Dukes' C. At one month post-resection, the PHI titer of six patients with colon cancer and of the patient with ulcerative colitis was £ 1:8. However, by four months post-resection, all but three of the patients had PHI titers in the positive range. These elevated titers were accompanied by recurrence of tumor growth and/or metastatic dissemination.

A radioimmunoassay was used to quantitate CEA in 22 of the sera which had been tested by PHI. When positive sera were defined as those which inhibited agglutination at dilutions of > 1:8 and contained CEA in excess of 5 ng per ml, the results of the two procedures were in agreement for 17 of the 22 specimens. Five sera, representative of two patients with colon cancer, were false negative by PHI.

Introduction

The carcinoembryonic antigen (CEA) was described, initially, as a cancer specific antigen peculiar to malignant tumors of entodermal origin and to the colonic mucosa of the two to seven month old fetus. Since that time, however, CEA and/or glycoproteins which cross-react...
immunologically with CEA have been detected in tumors of non-entodermal origin and in normal tissues of the adult.\textsuperscript{10,11,19}

In 1969, Thomson et al\textsuperscript{18} reported that CEA could be detected in the serum of patients with gastrointestinal cancer by using a technique of radioimmunoassay (RIA). The initial results of that test were exciting and the advent of new screening test for cancer seemed close to reality. Soon thereafter, however, other investigators reported that CEA could be found in the serum of patients with cancers of non-gastrointestinal origin and with non-malignant diseases of the gastrointestinal tract.\textsuperscript{14,15,16} Thus, it now appears that the RIA of CEA, in its present form, has no value as a screening test for cancer.

Several groups of investigators\textsuperscript{4,17} have suggested that the RIA for CEA can be useful for monitoring possible recurrence of tumor growth and/or metastatic dissemination following surgical therapy. These studies showed that successful ablation of the tumor was followed by a decline in levels of circulating CEA, whereas recurrence of tumor growth was accompanied by unaltered or increased levels.

In the current investigation, 11 patients with primary adenocarcinomas of the gastrointestinal tract and one patient with ulcerative colitis were studied post-operatively for changes in levels of serum CEA. In contrast to previous studies, wherein techniques of RIA were used for quantitating CEA, the present authors have used a technique of passive hemagglutination inhibition.\textsuperscript{1} This procedure, although slightly less sensitive than RIA, is very easy to perform. It is recommended for those clinical laboratories which are expected to conduct long-term follow-up studies involving large numbers of patients.

**Materials and Methods**

**Carcinoembryonic Antigen**

CEA was extracted from a pool of 10 primary adenocarcinomas of the colon. The purification procedure, which involved extraction of the tumor tissue with perchloric acid, and gel filtration on columns of Sepharose 4B and Sephadex G-200, has been described elsewhere.\textsuperscript{3,8}

**Anti-CEA Serum**

Pregnant goats were hyperimmunized, over a period of two years, with CEA recovered from the effluents of the Sephadex G-200 fractionation. The resultant antiserum was absorbed with perchloric acid extracts of normal colons and with packed human type AB-negative erythrocytes. Specificity of the antisera was confirmed by comparative immunodiffusion tests using goat anti-CEA sera.*

**Passive Hemagglutination Inhibition**

Procedures for the sensitization of erythrocytes with CEA and for the performance of the PHI titration were described in a previous report.\textsuperscript{1} In summary, duplicates of serial dilutions of the patients serum were examined for their capacity to inhibit the agglutination of CEA-sensitized cells in the presence of a predetermined amount of goat anti-CEA sera. Positive sera were defined as those which inhibited agglutination at dilutions of $>1:8$.

**Radioimmunoassay**

CEA was iodinated with $\text{I}^{125}$ by using the procedure outlined by Thomson et al.\textsuperscript{18} The capacity of the goat anti-CEA serum to bind 35 percent of 1 ng labeled CEA was supplied by Cordis Laboratories, Miami, FL and by Abbott Laboratories, North Chicago, IL.
CEA was determined by following the methods of Egan et al. A standard curve of inhibition was constructed by determining the capacity of known amounts of unlabeled CEA (1 to 1000 ng per ml) to inhibit the binding of 1 ng labeled CEA to the predetermined amount of antisera. The co-precipitation technique of Mac Sween et al. was then used to measure CEA levels in the serum specimens. Sera which contained more than 5 ng per ml were regarded as positive.

**Patients and Sera**

Pre-operative serum specimens were obtained from 7 patients with primary adenocarcinomas of the colon, three patients with primary adenocarcinomas of the rectum, one patient with a primary adenocarcinoma of the stomach and one patient with ulcerative colitis. The diagnosis was confirmed, in all cases, by pathological examination of a surgical biopsy.

Following surgical resection, the resected tissue was examined by staff pathologists and the adenocarcinomas of the colon and rectum were classified according to Dukes' method of staging. Post-operative sera were obtained one month after surgical resection and at monthly intervals thereafter for as long as contact with the patient could be maintained.

**Results**

In figure 1 is a composite of the results obtained when 12 patients were followed, post-operatively, for changes in levels of serum CEA. Whenever a sufficient volume of serum was available, it was assayed by both the PHI procedure and by the RIA. Results of the two procedures are compared in table I.

The pre-operative sera from nine patients with primary adenocarcinomas of the colon or rectum were positive by the PHI titration. When five of these sera were tested by radioimmunoassay, it was found that all contained CEA in excess of 10 ng per ml. The pre-operative serum from one patient (DS) with a primary adenocarcinoma of the colon in the stage of Dukes' C was negative by PHI and contained 7 ng CEA by RIA.

Because of concurrent medical problems, ablative surgery was not performed on EW. Her CEA level, as determined by both RIA and PHI, remained unchanged for one month following the initial assay.
TABLE I
Comparison of Passive Hemagglutination Inhibition
Titers with Levels of Carcinoembryonic Antigen
Determined by Radioimmunoassay

<table>
<thead>
<tr>
<th>Radioimmunoassay (ng per ml)</th>
<th>Reciprocal of PHI Titer</th>
<th>&lt;5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>&gt;30</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>5</td>
<td>11</td>
<td>16</td>
<td>21</td>
<td>26</td>
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<td>8</td>
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<td>128</td>
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<td>&gt;256</td>
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<td>Total</td>
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<td>6</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>22</td>
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</tbody>
</table>

Subsequently, EW developed an obstruction of the small intestine and a colostomy was performed. The patient exhibited a two-fold drop in titer immediately following the colostomy. However, at the time of the last assay, three months post-surgery, the PHI titer had reached 1:128. The prognosis of EW is listed as dismal.

Patients on whom ablative surgery was performed fell into several different categories with respect to the changes in CEA levels which occurred post-operatively. Two patients (EJ and SB) with primary adenocarcinomas of the colon in the stage of Dukes’ C failed to show a decline in serum CEA following resection. Sera from EJ, taken one month and six months post-operatively, exhibited PHI titers of > 1:256 and RIA values of > 100 ng per ml. These values remained unchanged until the time of death, seven months post-resection. Post-operative sera from SB inhibited agglutination at dilutions of 1:32 and contained 15 ng CEA per ml. SB expired three months post-surgery.

Patient EC exhibited a drop in PHI titer following ablative surgery but the titer did not fall below the lower limit of positivity. It was thought that EC was having a successful post-operative recovery until she was readmitted, six months later, with severe stenosis of her colostomy and hepatic metastases. Five days prior to death, her serum titered at 1:32 by PHI.

The one month post-operative sera from JG, AR, MM, DS, LL and SW were negative by PHI. The RIA results for JG, MM, and LL were also negative. MM is believed to be having a successful post-operative recovery; after six months, her PHI titer and RIA value remain unchanged. In contrast, the prognosis of JG, AR, DS, LL, and SW was considered poor. The two month post-operative serum of JG, who has metastatic carcinoma involving the lymphatics, had a PHI titer of 1:64. AR, whose primary lesion was non-resectable, had metastases to the ileum and the omentum. Sera obtained during the second month post-resection of the ileum had a PHI titer of 1:16 and an RIA value of 70 ng per ml. The primary tumor of DS could not be removed completely by surgery. Serum obtained during the second month post-resection was negative by PHI but contained 12 ng CEA per ml by RIA. DS expired four months post-resection. It was established that LL had a metastatic adenocarcinoma of the signet-ring type. Although his post-operative sera remained negative for two months, the PHI titer had increased to 1:64 by the fourth month. The pre-operative serum of SW had a PHI titer of 1:16 and contained 95 ng CEA per ml. Following surgical ablation her titer dropped to 1:8 and the RIA value was 30 ng CEA per ml. Serum obtained at two and three months post-resection were also negative by PHI. SW was transferred to a nursing home and additional sera were not available.

LG is a patient with primary adenocarcinoma of the stomach with metastases to the liver. His pre-operative serum had a PHI titer of 1:32. This titer remained stable for one month following gastrectomy, but after four months the titer was elevated at 1:128 and the RIA determina-
tion was 56 ng CEA per ml. The prognosis of LG is considered poor.

SZ is a young man who had a colon resection for ulcerative colitis. His preoperative serum had a PHI titer of 1:32. Follow-up sera obtained at one and three months post-resection have been negative by PHI and have contained < 2.5 ng CEA per ml.

Discussion

Several authors have reported that successful surgical ablation of a malignant tumor is followed by a decline in the level of circulating CEA. Conversely, persistence of high levels of CEA in post-operative sera has been associated with recurrence of tumor growth and/or metastatic dissemination. Thus, it now appears that assays of serum for CEA may be extremely valuable when attempting to evaluate the prognosis of the patient who has experienced surgery.

All previous studies dealing with the effects of surgical therapy on levels of circulating CEA involved the quantitation of CEA by RIA. These procedures are reported to be very sensitive and, providing that no modifications are introduced, very reproducible. However, most investigators will agree that the RIA of CEA is a very cumbersome procedure which requires a great deal of expertise. While the RIA may be suitable for industrial or research laboratories dealing exclusively with CEA assays, it is not commodius for the clinical laboratory which is expected to conduct long-term, post-operative, follow-up studies involving large numbers of patients. Thus, efforts must be directed toward the development of new and simple methods of CEA assay which can be incorporated into the many routine procedures which are part of the daily services of the hospital laboratory.

Lange et al. reported on the use of a passive hemagglutination inhibition test for detecting CEA in perchloric acid extracts of human serum. Subsequently, a similar PHI test, which utilized 25 µl untreated serum, was developed in our laboratory. The specificity of these PHI tests was comparable to the specificity of RIA procedures used by Moore and others. However, the sensitivity of the PHI test, as used in our laboratory, appears to be slightly less than that of both Lange's PHI and the RIA. This difference in sensitivity is probably due to the fact that Lange's test and the RIA utilize extracts derived from 5 ml and 1 ml of serum, respectively, while our PHI is performed on 25 µl untreated serum. The reproducibility of the PHI titration is very good, providing that care is taken in standardization of the protein content of the antigen preparation which is used for sensitization of the erythrocytes. In this respect, some investigators may dispute the applicability of the PHI test for CEA assays because of the large amounts of antigen (300 µg per ml) which are necessary for this sensitization.

The present investigation confirms that the PHI procedure can be used to monitor levels of CEA following surgical therapy. Elevated or rising PHI titers in post-operative sera were commonly associated with recurrence of tumor growth and/or metastases, whereas persistence of PHI titers of =≤ 1:8 were associated with successful post-operative recovery. It was found by us that when the preoperative titer was ≥ 1:32, the patient exhibited a drop in titer within one to three days post-surgery. Other investigators have suggested that this immediate drop in titer is due to rapid removal of CEA from the circulation by some patients. However, since many of these patients received from three to six units of blood during surgery, the present authors cannot exclude the possibility that transfusion contributes to the initial decline in titer. Consequently, these
early post-operative assays have not been included in our results.

Comparison of PHI titers with levels of CEA determined by RIA confirmed some of our earlier observations. All sera with PHI titers of $\geq 1:16$ contained CEA in excess of 10 ng per ml. Similarly, sera which contained less than 2.5 ng CEA per ml had PHI titers of $\leq 1:8$. However, five sera, representative of two patients (DS and SW) were negative by PHI but contained $> ng$ CEA per ml. Since these sera did not agglutinate the sensitized cells in the absence of the goat anti-CEA serum, no plausible explanation can be offered as to why such false negative reactions occur in some patients. Additional studies dealing with the quantitation of the PHI procedure are in progress and will be presented in a future report.

Summary

A technique of passive hemagglutination inhibition has been used to monitor post-operative sera for changes in levels of CEA. This technique is extremely easy to perform and, providing that an adequate supply of antigen is available, it can be incorporated into the routine procedures of any clinical laboratory without difficulty.

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References


