Radioimmuno-electrophoretic Binding Assay for the Detection of Carcinoembryonic Antigen

John A. Coller, Robert W. Crichtlow, and Lo Ke Yin
Hospital of the University of Pennsylvania, Department of Surgery and Harrison Department of Surgical Research, Philadelphia, Pennsylvania

SUMMARY

A new radioimmunoassay (RIA) method has been described. This radioimmuno-electrophoretic binding (REB) technique has been applied to the carcinoembryonic antigen system. In contrast to the RIA method with the use of perchloric acid extraction, in the REB technique the requirement for lengthy and possibly biologically degrading procedures on relatively large volumes of sera has been eliminated. Only one-hundredth as much serum is required by REB than is required by RIA, and the assay can be completed in 2 hr rather than in 5 days. While the sensitivity of REB in the detection of serum carcinoembryonic antigen is similar, there appears to be a different but related specificity, compared to the RIA method.

INTRODUCTION

The design of any RIA3 system is dictated by the immunochemical characteristics of its components. In general, there are two main parts, a sequential series of incubations to effect antigen-antibody binding and a means of separation of bound from unbound labeled reactant. Frequently, chromatographic electrophoresis is used in the separation of the unreacted, labeled moiety from antigen-antibody complexes. However, electrophoresis has not been utilized in RIA systems as a means of promoting the initial binding. This paper describes a method whereby the entire process of binding and separation is carried out simultaneously in an electrophoretic field. This method has been termed REB4 and has been applied to the detection of CEA in human sera.

The CEA is a glycoprotein of β-electrophoretic mobility, described by Gold and Freedman (3). It is isolated from primary or metastatic adenocarcinomatous tissue originating from colonic mucosa. In 1969, Thomson et al. (10) described a RIA procedure for the detection of CEA in human sera. This 5-day method involves the perchloric acid extraction of 5.0 ml of serum, centrifugation, dialysis, lyophilization, resuspension in antibody-containing solution, incubation with labeled antigen, and centrifugal separation of bound labeled antigen with ammonium sulfate. In contrast, the REB method described here involves simultaneous electrophoretic binding and separation with the use of 10 µl of whole sera and requires only about two hours.

MATERIALS AND METHODS

Antigen. To facilitate comparison of the REB and RIA methods, we used the same antigen and heterologous antibody preparations for both assays. These materials were provided by Dr. Phil Gold of Montreal, Canada. Briefly, the isolation of CEA is accomplished by sequential Sepharose 4B, Sephadex G-200 column-gel filtration and Sephadex block electrophoresis of a 1.0 M perchloric acid extract of colonic adenocarcinoma hepatic metastatic tumor tissue (7). “Purified” CEA is labeled with 125I by a modification of the chloramine-T method of Greenwood et al. (5).

Antibody. Heterologous anti-CEA antibody produced in goats was absorbed with normal human plasma and normal colonic mucosal extracts. Absorbed antisera gave a single band of immunoreactivity by Ouchterlony double-gel immunodiffusion against colonic carcinoma mucosal extracts but did not give a band with extracts of normal mucosa. Dilutions of reactants were made in 0.05 M borate buffer, pH 8.6, containing 1:20 normal human sera.

Electrophoresis. Gel plates were made on glass lantern slides (3.25 x 4.25 inch) with agarose (1.1%) in barbital buffer, 0.037 ionic strength, pH 8.2 (1). A gel cutter was constructed that provided 3 reactant wells (3 mm in diameter) along the electrophoretic axis (Chart 1). Between each row of reactant wells, 2 larger wells were provided. The larger wells, left void during electrophoresis, assist in maintaining horizontal alignment of the electrophoretic current and minimized lateral dispersion of protein flow. Electrophoresis was performed with barbital buffer, 0.037 ionic strength, pH 8.2, at 80 V, 40 to 45 ma for 90 min. At the completion of electrophoresis, the gel was sectioned so that for each row of reactant wells the radioactivity of the anodal segment could be compared to that which remained within the cathodal segment. A γ count of the gel sections was performed in a Packard Model 578 γ counter with a 3-inch sodium iodide crystal.

Basic Format of REB. The electrophoretic conditions are such that, at the completion of electrophoresis, CEA125I originating in Well A will have migrated past Wells B and C to...
Chart 1. Agarose-coated glass slide after application of gel cutter. The 10-μl volumes of reactants are placed as follows: Well A, CEA-125I; Well B, unlabeled CEA or human sera; Well C, heterologous anti-CEA antibody. For each row of reactant wells at completion of electrophoresis, the gel is sectioned (-----) so that the radioactivity in the anodal segment can be compared with that of the cathodal segment.

the anodal segment, if reactants in Wells B and C do not impede its migration. If specific antibody is placed in Well C, its cathodal migration will result in the binding of a portion of the CEA-125I between Wells A and C, causing decreased anodal migration of labeled antigen. The amount of labeled antigen bound is a function of the strength of the antibody concentration. If a constant concentration of antibody is placed in Well C and unlabeled antigen is placed in the intervening Well B, then, during electrophoresis, antibody will first encounter and in part be bound by unlabeled antigen before exposure to labeled antigen. The result will be a decrease in antibody available to bind CEA-125I, as indicated by increased migration of radioactivity to the anodal segment. A schematic representation of the electrophoretic reaction is shown in Chart 2.

RESULTS

Electrophoretic Migration of CEA-125I. CEA-125I (10 μl with about 10,000 cpm) is placed in Well A and buffer is placed in Wells B and C. At the completion of electrophoresis, 85 to 95% of the radioactivity is recovered in the anodal segment.

Electrophoretic Binding of CEA-125I. Serial dilutions (10 μl) of absorbed goat anti-CEA antibody is placed in Well C and buffer is placed in Well B. Upon completion of electrophoresis, with greater antibody there is a decrease in radioactivity recovered from the anodal segment. The relationship of antibody concentration to electrophoretic migration of CEA-125I is shown in Chart 3.

Inhibition of Electrophoretic Binding of CEA-125I by Unlabeled CEA. From the electrophoretic binding curve, an antibody dilution is selected that results in the binding of about 65% of the CEA-125I (35% migration to the anodal segment). This dilution (10 μl of 1:800, shown in Chart 3) is designated the "standard antibody" and is placed in Well C for all subsequent experiments. Varying concentrations of unlabeled purified CEA are placed in Well B. As increasing concentrations of unlabeled CEA are used, a greater proportion of CEA-125I migrates to the anodal segment (Chart 4). Approximately 3 ng of unlabeled CEA is required to cause significant inhibition of the electrophoretic binding of CEA-125I. As indicated by the migration of 85% of the radioactivity to the anodal segment, 25 ng of unlabeled CEA effectively binds all of the standard antibody.

REB of Human Sera. For the testing of human sera, 10 μl of whole sera are placed in Well B, and the standard antibody dilution is placed in Well C. Each assay is performed in duplicate along with control normal human sera and a known positive. Although both REB and RIA assays may be interpreted quantitatively, these results will be discussed only in terms of being positive or negative. A specimen is designated positive by REB if the percentage of radioactivity migrating to the anodal segment minus 2 S.D. of the duplicate mean of the specimen is 5% greater than the control plus 2 S.D. of the duplicate mean of the control. A specimen is positive by RIA if the precipitable radioactivity of the duplicate mean of the specimen is 10% less than that of the control. A comparison of the results of REB and RIA for 122 patients is given in Table 1.

DISCUSSION

Comparison of REB and RIA in the Detection of CEA in Human Sera. In all disease categories, there were as many or slightly more positives by RIA than by REB. Fifty-five and 63 specimens were positive by REB and RIA, respectively. If this
Chart 3. Electrophoretic binding of CEA-\(^{125}\)I. A constant volume of CEA-\(^{125}\)I is placed in Well A. A constant volume of heterologous anti-CEA antibody of increasing dilution is placed in Well C, and buffer is placed in Well B.

![Chart 3](chart3.png)

Chart 4. Inhibition of electrophoretic binding by unlabeled CEA. A constant volume of CEA-\(^{125}\)I is placed in Well A. A constant volume of a 1:800 dilution of heterologous anti-CEA antibody is placed in Well C. A constant volume of purified CEA of increasing concentration is placed in Well B.

![Chart 4](chart4.png)

Although investigations of this new technique of radioimmunoassay have not yet been sufficient to determine the nature of the disparity between the 2 methods, it is evident that the marked contrast in methodology probably plays a fundamental role (Table 2). In contrast to the REB system, in which unprocessed whole serum is used, the RIA system uses a strong concentration of perchloric acid (1.0 M) to extract the CEA-containing glycoprotein fraction from the serum. The use of such a potent oxidizing agent may very well structurally alter the moiety being detected and (although leaving it physiochemically intact) alter its immunoreactivity. Presumably, perchloric acid exposure has been deleterious to any coexisting antibody or naturally occurring antigen-antibody complexes of CEA that might be present in the serum specimens. Since there is no extractive preparation of specimens when the REB assay is used, it might be expected that additional antibody (if it exists) contributed by the test serum could interfere with the inhibition of electrophoretic binding. The possibility of autogenous antibody interference has been evaluated by subjecting the test specimen to electrophoresis against labeled antigen in the absence of heterologous antisera. Under the electrophoretic conditions used, no significant evidence of free or complexed autogenous antibody activity was demonstrated.

Recently, Gold et al. (4) demonstrated evidence by autoradiography of IgM binding with CEA-\(^{125}\)I that had previously been incubated with human serum containing anti-A isoantibody. We have not noticed a similar relationship of binding with blood group substances as measured by REB. Gold used electrophoresis only for the separation, after incubation, of bound from unbound labeled antigen and not for the promotion of the initial antigen-antibody reaction, as is done with REB. This may in part account for the observed difference.

In contrasting the RIA and REB methods, antigen-antibody
Table 1
Comparison of REB and RIA in the detection of CEA in human sera

<table>
<thead>
<tr>
<th>Specimen</th>
<th>No. of patients</th>
<th>REB+</th>
<th>REB+ and RIA+</th>
<th>REB+ and RIA-</th>
<th>REB- and RIA+</th>
<th>REB- and RIA-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>28</td>
<td>19</td>
<td>21</td>
<td>17</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Pancreas</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Breast</td>
<td>17</td>
<td>11</td>
<td>13</td>
<td>10</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Lung</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>26</td>
<td>10</td>
<td>13</td>
<td>5</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Subtotal</td>
<td>77</td>
<td>46</td>
<td>53</td>
<td>38</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Nonmalignant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon polyp</td>
<td>12</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Other</td>
<td>33</td>
<td>8</td>
<td>8</td>
<td>1</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Subtotal</td>
<td>45</td>
<td>9</td>
<td>10</td>
<td>1</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

* REB+ and REB-, specimens positive and negative, respectively, by REB assay; RIA, specimens positive and negative, respectively, by RIA assay.

Prostate, testicular, ovarian, vaginal, cervical, transitional cell, clear cell, cutaneous, Hodgkin's, reticulum cell, unknown primary.

Table 2
Contrast of REB and RIA methodology

<table>
<thead>
<tr>
<th>Assay</th>
<th>Specimen</th>
<th>Volume (ml)</th>
<th>Extraction</th>
<th>Immunoreaction</th>
<th>Separation</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIA</td>
<td>Serum</td>
<td>5.0</td>
<td>Perchloric acid precipitation; centrifugation; dialysis; lyophilization</td>
<td>Dissolve in antibody solution; incubate; add CEA-125 1; incubate</td>
<td>50% (NH4)2SO4; centrifugation; aspiration; y counter</td>
<td>5 days</td>
</tr>
<tr>
<td>REB</td>
<td>Serum</td>
<td>0.010</td>
<td>None</td>
<td>Subject to electrophoresis with CEA-125 1; antibody</td>
<td>Cut gel; y counter</td>
<td>2 hr</td>
</tr>
</tbody>
</table>

Table 3
Comparison of REB and RIA in the detection of malignant disease

<table>
<thead>
<tr>
<th>Carcinoma</th>
<th>REB</th>
<th>RIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Colon</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td>Breast</td>
<td>9</td>
<td>36</td>
</tr>
<tr>
<td>Other</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>All</td>
<td>46</td>
<td>29</td>
</tr>
</tbody>
</table>

Comparison of REB and RIA in the Detection of Cancer. A comparison of CEA association with disease groups as detected by each method is shown in Table 3. Although all the pancreatic and pulmonary carcinomas are positive by both methods, the small sampling precludes separate statistical interpretation. The association of CEA with carcinoma of the colon or breast as detected by either test is highly significant (p < 0.001).

The nonmalignant category, comprising 45 patients, was not...
John A. Coller, Robert W. Crichlow, and Lo Ke Yin

a “young, healthy control” group. All were hospitalized patients, most of whom had symptoms requiring gastrointestinal evaluation. In general, a similar pattern of a rather wide distribution of detectable levels of CEA among cancer-bearing patients and among a significant percentage of the nonmalignant population has been reported by Lo Gerfo et al. (8) and Moore et al. (9). These findings have been obtained with the RIA method, as well as with the zirconyl phosphate gel method described by Hansen et al. (6). It appears that, as with the zirconyl gel method, REB has a different but related specificity, compared to RIA.

REFERENCES

Radioimmunoelectrophoretic Binding Assay for the Detection of Carcinoembryonic Antigen

John A. Coller, Robert W. Crichlow and Lo Ke Yin


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/33/7/1684

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.