Adjuvant to the Diagnostic Distinction between Adenocarcinomas of the Ovary and the Colon Utilizing a Monoclonal Antibody (COL-4) with Restricted Carcinoembryonic Antigen Reactivity

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ABSTRACT

Malignant ovarian tumors may represent either primary ovarian cancers or metastatic lesions (from patients with demonstrated primary cancers at other body sites) whose distinction may be difficult using clinical, surgical, and pathological criteria. Monoclonal antibody (MAb) COL-4, reactive with carcinoembryonic antigen, has previously been shown to react preferentially with adenocarcinomas of the colon versus a variety of normal tissues. We report here that MAb COL-4 is strongly reactive with primary colon carcinomas (N = 50), as well as regional (N = 42) and distant (N = 20) metastases of colonic adenocarcinoma. In contrast, MAB COL-4 demonstrated little to no reactivity with primary (N = 53) and metastatic carcinomas of the ovary (N = 23) including serous, mucinous, and poorly differentiated adenocarcinomas using immunohistochemical techniques. This differential reactivity was statistically significant (P < 0.001), suggesting the potential clinical utility of MAB COL-4 in the differentiation of ovarian from colonic adenocarcinoma. Solid-phase quantitative radioimmunoassays and Western blotting techniques confirmed these results. Data are also presented that the carcinoembryonic antigen molecules or epitopes recognized by a more classical broadly reactive anti-carcinoembryonic antigen MAb are distinct from those recognized by MAB COL-4. Other carcinomas which also metastasize to the ovary and may be confused clinically with a primary ovarian tumor such as adenocarcinomas of the stomach and breast were also evaluated for reactivity with MAB COL-4. COL-4 was reactive with all gastric carcinomas evaluated, but failed to react with breast carcinomas. Hence, COL-4 can now be utilized as an immunohistochemical adjunct for the differentiation of ovarian from gastrointestinal adenocarcinoma which can be difficult to distinguish by clinical, surgical, and histological parameters.

INTRODUCTION

Adenocarcinomas arising from the large intestine and ovary rank third and fifth, respectively, as the causes of cancer mortality among American women (1). Ovarian tumors may represent either primary ovarian carcinomas or metastatic lesions from primary cancers at distant body sites. This diagnostic dilemma is at times difficult to resolve using clinical, surgical, and pathological criteria (2-6). As a result, there is considerable controversy over the incidence of metastatic tumors in the ovary, with figures varying from 6% (7) to 61.5% (4). Cancers which commonly metastasize to the ovary include tumors of the breast (41%), gastrointestinal tract (37%), genital tract (22%), and hematopoietic system (3, 8, 9). The surgical, therapeutic, and prognostic implications of each tumor type are different, hence the ability to make this distinction is of tantamount clinical importance.

Gastrointestinal adenocarcinomas which may metastasize to the ovary and mimic primary ovarian cancer include, in order of decreasing frequency, gastric, colorectal, and biliary tract carcinomas. Metastatic tumors from the colon may be particularly difficult to distinguish from ovarian primary carcinomas, because both may be solid or cystic, unilateral or bilateral, show considerable hemorrhage and necrosis, and attain a tremendous size weighing 2000 g or more completely overshadowing a primary gastrointestinal tumor (2). Mucin-producing colonic adenocarcinomas, as well as mucinous cystadenocarcinomas of the ovary, may be lined by an “intestinal type” of epithelium. Metastatic colonic carcinomas are usually composed of well-formed neoplastic glands, with or without dilated intraluminal spaces containing mucinous material. Furthermore, in some cases they may simulate hormonally active ovarian tumors with luteinization of the surrounding stroma, subsequent hormone production, masculinization, and other endocrine changes (3, 10). Ovarian mucinous cystadenocarcinomas often present similar clinical as well as pathological findings, i.e., they may be unilateral or bilateral, produce mucinous secretion with resultant cystic dilatation of glandular spaces, and usually are lined by a “picket fence” arrangement of epithelial cells which may include goblet or argentaffin cells commonly found in the intestinal epithelium. Intestinal enzyme production by the malignant ovarian epithelial cells has also been reported; enzymes produced include lipase, trypsin, amylase, and succrase (10).

Antigenic cross-reactivity between adenocarcinomas of the colon and ovary using antibody probes directed against histocompatibility antigens, tumor-associated antigens (11-16), and oncofetal antigens (17, 18) has been documented. CEA, an extensively studied complex glycoprotein with a molecular weight of 180,000 has been described in embryonic colonic mucosa and adenocarcinomas of the colon (19-22), ovary (23, 24), and breast (25). CEA expression has been assayed using a number of antibody preparations in ovarian tumors, and has been particularly associated with epithelial tumors of the mucinous type (26, 27). Circulating plasma levels of CEA have also been evaluated in patients with ovarian carcinomas (28, 29), and have generally been higher in patients with mucinous cystadenocarcinomas (53%) in comparison to patients with serous cystadenocarcinomas (31%). However, significant plasma levels of CEA have also been detected in 15% of patients with benign gynecological diseases (18) and other disorders (e.g., alcoholic liver disease (30, 31) and inflammatory bowel diseases (32)), limiting the diagnostic utility of plasma CEA levels for detecting occult carcinoma, determining histological tumor type, differentiating benign from malignant lesions, or discriminating primary from metastatic tumors.

[The abbreviations used are: CEA, carcinoembryonic antigen; MAb, monoclonal antibody; PBS, phosphate buffered saline (80 mm Na₂HPO₄, 1.5 mm KH₂PO₄, 2.5 mm KCl, 140 mm NaCl, pH 7.4); BSA, bovine serum albumin; NCA, normal cross-reacting antigen; G anti-Mu IgG, goat-anti-mouse immunoglobulin G; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.]
Numerous reports have indicated that “CEA” may indeed be a family of isoantigens rather than a single antigen. In addition, it has been demonstrated that anti-CEA antibodies may differ dramatically in their reactivities (33, 34). Conventional hyperimmune antisera raised against CEA react with many different types of carcinomas and antigenic determinants expressed in normal tissues (35–43). Monoclonal antibodies reactive with CEA have also been shown to react with CEA-related antigens in normal colonic mucosa (44), normal spleen (45, 47–51), normal liver (47, 52), normal lung (47), sweat glands (51), polymorphonuclear leukocytes (44, 45, 47, 50–53), and monocytes (45). The CEA cross-reacting antigens most commonly described include: NCA (35–38), NCA-2 (37–39, 41), normal fecal antigen (43), meconium antigen (54), and biliary glycoprotein (42).

We have recently generated MAbs, designated COL-1 through COL-15, which recognize at least five different epitopes of the CEA molecule. These MAbs were shown to be preferentially reactive with carcinomas of the colon when compared to numerous normal adult human tissues (34). As a follow-up to these studies (34), the COL MAbs were assayed for reactivity with other carcinomas. It was noted that COL-4 had a rather restricted range of reactivity, including primarily colonic carcinomas. Therefore, this study was initiated to (a) further define the cellular expression of the CEA epitope recognized by COL-4 in primary and metastatic ovarian and colonic carcinomas; (b) determine whether COL-4 immunoreactivity with ovarian tumors differs from that previously reported for other anti-CEA antibodies; (c) compare the immunoreactivity of COL-4 and a broadly cross-reactive anti-CEA MAb B1.1 (25, 34, 51) with adenocarcinomas of the colon, ovary, breast and stomach; and (d) investigate the potential usefulness of MAb COL-4 reactivity as an immunohistochemical marker to help differentiate ovarian carcinoma from adenocarcinoma of the gastrointestinal tract.

MATERIALS AND METHODS

Surgical Tissue Specimens. Paraffin blocks containing formalin-fixed, surgically resected gastric, colonic, breast, and ovarian tumors (primary and metastatic) were obtained from the Departments of Surgery or Surgical Pathology, Vanderbilt University Hospital, Nashville, TN; Duke University Medical Center, Durham, NC; and George Washington University Medical Center, Washington, DC. Each tumor utilized for this study was classified by each pathology department at the time of surgical resection as to the histological tumor type and organ of most probable origin using clinical, surgical, and histological data. Primary colorectal carcinomas were each demonstrated to arise from the gastrointestinal epithelium, hence the diagnosis was certain. Metastatic colon carcinomas were histologically compatible with a primary adenocarcinoma of the colon, and occurred either concomitant with or subsequent to a primary colon cancer resection. Primary serous ovarian tumors were clearly diagnosed as such because of a distinct histological appearance. Mucinous tumors occurring in the ovary were diagnosed as primary lesions if they were grossly and histologically compatible with that diagnosis and they occurred in patients with no other known primary adenocarcinomas. These lesions, as well as endometrioid tumors of the ovary, may be difficult to distinguish from metastases on purely histological grounds as discussed. Metastatic tumors from the ovary were diagnosed on the basis of histological compatibility with the primary tumor. Five-µm-thick sections were generated and mounted on gelatin-coated slides.

Monoclonal Antibodies. Monoclonal antibody COL-4 was generated using extracts of two colon carcinoma metastases (one from lymph node and the other from spleen) and an extract of a primary colon carcinoma as sequential immunogens in BALB/c mice as previously described (34). Somatic cell hybrids were cloned twice and an immunoglobulin designated COL-4 was identified. The isotype IgG2a was determined using previously published methods (34). A single pool of hybridoma tissue culture supernatant was used for all experiments; two subsequent pools were evaluated and found to be similar using immunohistochemical analyses. An isotype identical control MAb UPC-10 (55) (8 µg/200 µl; Litton Bionetics, Charleston, SC) and supernatant fluid from the parent nonsecreting mouse myeloma cell line (NS1) were used as negative controls for all tissue samples. All controls were performed simultaneously on adjacent serial tissue sections; no reactivity was observed. MAb B1.1 (25, 33, 34, 56) has previously been shown to be broadly reactive with CEA (M, 180,000) and CEA-related molecules (approximately M, 70,000–90,000).

Immunohistochemical Methods. Formalin-fixed, paraffin-embedded human tumors were assayed for antigen expression using a modification of previously published methods (57). Briefly, tissue sections were deparaffinized in xylene, rehydrated in graded alcohols, and endogenous peroxidase activity was blocked using methanol containing 0.3% H2O2 for 15 min at room temperature. After rinsing in PBS, the sections were incubated in 10% normal horse serum for 15 min. This and all subsequent reagents were diluted in PBS containing 0.1% BSA and added at 200 µl/slide. The pretreatment serum was removed and primary MAbs COL-4 (undiluted tissue culture supernatant), UPC-10 (8 µg/200 µl), or NS-1 tissue culture supernatant were added for 30 min at room temperature. After the primary MAbs were removed, the sections were rinsed in PBS and then incubated with biotinylated horse anti-mouse immunoglobulin (Vector Laboratories, Inc., Burlingame, CA). The slides were again rinsed in PBS, then incubated for 30 min with avidin-biotin-horseradish peroxidase H complex (ABC Kit; Vector) at room temperature. Following another rinse, 0.06% diaminobenzidine (Sigma) with 0.01% H2O2 was added and the slides were briefly counterstained with hematoxylin, dehydrated in graded alcohols, cleared in xylene, mounted under a coverslip, and examined using a light microscope.

Immunohistochemical Scoring Methods. Each section was evaluated for the presence of epithelial intracellular (apical or cytoplasmic), stromal, or extracellular reddish-brown diaminobenzidine precipitate indicative of MAb binding. Faint reactivity not visible using a ×40 magnification was considered negative. Sections were scored + for clearly positive and ++ for dark brown reactivity. The sum of + and ++ reactivities was utilized for total reactivity. An approximate percentage of positive malignant ovarian and colonic epithelial cells was assigned according to the number of malignant cells positive divided by the total number of malignant epithelial cells ×100. Benign tissues and cell types were not scored, although they were generally nonreactive. Stromal cells were negative in all cases. Serial sections of colonic adenocarcinoma known to be reactive with COL-4 were included in each assay to ensure interassay reproducibility. For the primary and metastatic lesions (regional and distant), each was assigned an independent score based on the reactivity of MAb COL-4 with one to two representative tissue samples (paraffin-embedded blocks).

Cell Extract Preparation. Surgically resected primary and metastatic ovarian and colon tumors (snap frozen, stored at −70°C) were obtained from the Departments of Surgery and Pathology, George Washington University Hospital, Washington, DC; Department of Pathology, Vanderbilt University Hospital, Nashville, TN; and Department of Pathology, Duke University Medical Center, Durham, NC. Nine cell extracts were prepared as previously described (34). Briefly, tissues were minced finely and homogenized for 2–3 min at 4°C in 10 mm Tris-HCl buffer, pH 7.2, 0.2 mM CaCl2 (at a ratio of 1 g tissue to 10 ml buffer). The homogenate was subjected to nitrogen cavitation using a cell disruption bomb (Parr Instrument Co., Moline, IL) at 1,000 psi, and then clarified at 1,000 x g for 5 min. The supernatant was sonicated at 4°C for 1 min in 15-s intervals (Branson Sonifier, setting 7). The sonicate was then centrifuged at 10,000 x g for 10 min, and the supernatant was used for solid-phase radioimmunoassays and Western blotting. Protein concentrations were determined by the method of Lowry et al. (58). All tissues were coded such that the pathological diagnosis was unknown at the time of analysis.

Solid-phase Radioimmunoassays. Fifty µl containing serial dilutions

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of cell extract (from 5 μg to 0.07 μg) were added to each well of 96-well polyvinyl microtiter plates and allowed to dry overnight at 37°C in a nonhumidified incubator. The wells were quenched to minimize nonspecific protein binding by the addition of 100 μl of 5% BSA in phosphate buffered saline containing calcium and magnesium (PBS with Ca2+ and Mg2+). The BSA was aspirated after an incubation of 1 h at 37°C, and 50 μl of undiluted COL-4 or B1.1 tissue culture supernatant were added to each well. After 1 h of incubation the unbound immunoglobulin was removed and the plates were washed with 1% BSA in PBS. Each well was then incubated for 1 h at 37°C with 125I-labeled G anti-Mu IgG (Kirkegard and Perry, Gaithersburg, MD) at 75,000 cpm in 25 μl in order to detect the specifically bound immunoglobulin. The unbound 125I anti-Mu IgG was aspirated and the wells were washed extensively with PBS and 1% BSA. The specifically bound 125I anti-Mu IgG was detected by cutting the individual wells and measuring the radioactivity in a gamma counter.

Western Blotting. Forty μg of each cell extract was diluted in SDS-PAGE sample buffer (0.125 M Tris-HCl, pH 6.8, 4% SDS-20% glycerol, 10% 2-mercaptoethanol), loaded and analyzed using a 5–20% linear gradient SDS-PAGE. After electrophoresis, proteins were transferred to nitrocellulose paper (0.45-μm pore size) at 4°C for 4 h at 30 V in transfer buffer (25 mM Tris-HCl, pH 8.3, 192 mM glycine, 20% methanol). The blots were then incubated in PBS containing 5% BSA for 2 h at room temperature and then washed with PBS containing 0.05% Tween-20. Ten μl of hybridoma tissue culture supernatant of either COL-4 or B1.1 were added, and incubated for 2 h at room temperature with gentle agitation. After washing with PBS containing 0.05% Tween-20, the blots were incubated with 125I anti-mouse IgG for 1 h at room temperature. The nitrocellulose filters were then washed overnight and exposed to Kodak XAR-5 X-ray film with a DuPont Lightning Plus intensifying screen at −70°C for at least 2 h. For each experiment NS-1 tissue culture supernatant was used as a negative control.

RESULTS

MAB COL-4 Reactivity with Colon Carcinomas. Immunohistochemical methods were used to determine the reactivities of MAB COL-4 with tumors diagnosed as (a) primary colon carcinoma, (b) metastatic colon carcinoma, (c) primary ovarian carcinoma, or (d) metastatic ovarian carcinoma. Forty-nine of 50 adenocarcinomas of the colon were immunoreactive with COL-4, although heterogeneity in the percentage of malignant cells staining was noted in virtually all 49 primary tumors (Fig. 1A). Forty of 42 regional metastases (Fig. 1B), and 19 of 20 distant metastases (including six metastases to the ovary which were histologically compatible with metastatic colon carcinoma, Fig. 1C) were also immunoreactive. For each of these categories the range of reactivities varied, from no reactivity to almost 100% reactivity of the malignant colonic epithelial cells. Generally, no difference in COL-4 antigen expression was noted between superficial or deeply invasive cancers. In addition, tumor differentiation and mucin production were not factors in COL-4 binding. Intracellular cytoplasmic MAB binding as well as extracellular immunoreactivity (within the extracellular mucin-like material) was observed (Fig. 2A).

MAB COL-4 Reactivity with Ovarian Carcinomas. In general, MAB COL-4 demonstrated no or weak immunoreactivity with 73 common epithelial tumors of the ovary, including serous cystadenocarcinomas, mucinous cystadenocarcinomas, poorly differentiated carcinomas, clear cell, and endometrial carcinomas (Fig. 1, D and E). Twenty-eight primary serous cystadenocarcinomas were all negative for COL-4 reactivity with the exception of one tumor which was ≤1% reactive. Of the histological subtypes classified as ovarian tumors mucinous cystadenocarcinomas were generally the most immunoreactive (seven of 13 primary and five of five metastatic tumors demonstrated at least one cell reactive with COL-4). However, only one of 13 primary and one of five metastatic mucinous tumors were ≥10% reactive. Immunohistochemical staining of these tumors was generally focal, cytoplasmic, and membranous (Fig. 2C). Extracellular staining of the mucin-like material was rarely observed. Serous cystadenocarcinomas were generally nonreactive with COL-4 with ≤1% cellular reactivity in one of 28 primary and one of 16 metastatic tumors (Fig. 2D). Poorly differentiated ovarian carcinomas were uniformly nonreactive (five primary and two metastatic) with COL-4. One primary endometrial ovarian carcinoma (Fig. 1D) demonstrated ≤5% cells immunoreactive. Of the 23 metastatic ovarian tumors examined, seven were surgically resected from the large or small intestine, and demonstrated 0% to ≤5% cellular reactivity with MAB COL-4.

Statistical Analysis of Immunohistochemical Results. Contingency analysis, χ2 x 2, of COL-4 reactivity with ovarian and colon carcinomas was performed using standard methods (59); the results are listed in Table 1. The independent samples were analyzed for any immunoreactivity with MAB COL-4 (i.e., ≥1 cell reactive). Of particular importance was a statistical comparison of COL-4 reactivity with primary colon carcinomas and primary serous ovarian tumors, as their diagnosis is nearly certain and thus these two groups serve as our excellent controls. As seen in Table 1, COL-4 reactivity with primary colon carcinomas versus primary serous ovarian tumors was statistically significant (P < 0.001). Based on these data, COL-4 immunoreactivity of malignant epithelial cells strongly supports a diagnosis of gastrointestinal adenocarcinoma rather than primary ovarian cancer. A comparison of experimental groups including metastatic colon carcinomas, mucinous, or poorly differentiated ovarian carcinomas also shows that with these specific tumors, COL-4 reactivity supports a diagnosis of a colon metastasis rather than an ovarian primary (P < 0.005). The reverse scenario is also true, as a comparison of COL-4 reactivity with primary colon carcinomas and metastatic ovarian carcinomas yields a P value of <0.001.

Comparison of MABs B1.1 and COL-4. The anti-CEA MAB B1.1 has previously been shown to be reactive with human colon and breast carcinomas, with a reactivity similar to most anti-CEA MABs reported in the literature (51). MAB B1.1
reacts with the $M$, 180,000 CEA molecule within cells as well as other CEA-related lower molecular weight species. The reactivities of the two MAb COL-4 and B1.1 with, respectively, restrictive and nonrestrictive anti-CEA MAbs (34) were compared using immunohistochemical techniques on serial tissue sections of colon, ovarian, and breast carcinomas (Table 2, Fig. 2). Briefly, both MAbs demonstrated similar reactivities with adenocarcinomas of the colon ($N = 4$), although MAb B1.1 reacted with a higher percentage of the malignant epithelial cells than COL-4 (average reactivities of 75 and 63%, respectively). The cellular distribution of immunoreactivity with each MAb was generally indistinguishable (Fig. 2, A and B).

Ovarian carcinomas were more strongly reactive with the B1.1 anti-CEA antibody. MAb B1.1 was strongly reactive with three of five mucinous tumors (85, 95, and 75% cellular reactivity) and weakly reactive with a fourth (Table 2). In contrast, MAb COL-4 showed only trace reactivity with one of five of these tumors. Both MAbs were completely nonreactive with four serous cystadenocarcinomas.

**Breast and Stomach Carcinoma.** Adenocarcinomas of the breast and stomach were also evaluated for their reactivities with COL-4 and B1.1 because they must be considered in the differential diagnosis of an ovarian mass. Invasive ductal carcinomas of the breast were similar to ovarian tumors in that they demonstrated a differential reactivity when employing MAbs COL-4 and B1.1. Three of five tumors were moderately reactive with B1.1, whereas only one of five tumors was focally reactive with COL-4 (Table 2, Fig. 2, G and H). In contrast, four of four gastric adenocarcinomas were strongly reactive with both anti-CEA MAbs COL-4 and B1.1 (Table 2).

**Solid-phase Radiimmunoassay for CEA.** The differential reactivities of MAbs COL-4 and B1.1 were quantitated in solid-phase radiimmunoassays with cell extracts of primary ovarian cancer, primary colonic carcinoma, and adenocarcinomas of the colon metastatic to the ovary. This assay was employed to further evaluate differences in levels of expression of each CEA epitope (recognized by MAbs B1.1 and COL-4) and to rule out the possibility that immunohistochemical results described above might be the result of "artifactual" antigenic denaturation due to tissue fixation and processing.

As shown in Fig. 3A all six primary ovarian carcinomas failed to react with MAb COL-4. Two of these six tumors, on the other hand, reacted with MAb B1.1 (Fig. 3B). Primary adenocarcinoma of the colon, in contrast, demonstrated reactivity with both MAbs COL-4 and B1.1 (Fig. 4, A and B). Two tumors from the ovary, believed to be metastatic colon carcinomas, resected from patients with primary adenocarcinoma of the colon with demonstrated metastases, were also evaluated for CEA expression using COL-4 and B1.1. As shown in Fig. 4, A and B, both tumors reacted equally well with MAbs COL-4 and B1.1, similarly to the primary adenocarcinoma of the colon. These results were markedly different from those obtained with the primary ovarian tumors (Fig. 3, A and B). Although these tumors were suspected clinically to be metastatic colon carcinoma at the time of surgical resection, a definitive pathological diagnosis of primary ovarian versus metastatic adenocarcinoma had not been rendered because each was histologically consistent with either a primary mucinous cystadenocarcinoma or a metastatic adenocarcinoma of the colon. The reactivity of these tumors with COL-4 is consistent with a diagnosis of metastatic adenocarcinoma of the colon to the ovary in each case.

**Western Blotting.** Extracts of primary ovarian and colon carcinomas, as well as a tumor clinically consistent with a metastatic colon carcinoma metastases (Fig. 5B, lanes 1 and 3), were evaluated for CEA expression using COL-4 and B1.1. As seen in Fig. 5A, COL-4 immunodetected a $M$, 180,000 protein characteristic of the CEA molecule both in the primary and suspected metastatic colon carcinoma (lanes 1 and 3), but was nonreactive with the primary ovarian carcinoma (lane 2).

MAb B1.1 recognized a $M$, 180,000 band in the primary colon and suspected colon carcinoma metastases (Fig. 5B, lanes 1 and 3). However, in the primary ovarian carcinoma it was immunoreactive with a lower molecular weight protein (Fig. 5B, lane 2). This lower molecular weight molecule is believed to be a CEA cross-reacting antigen (33, 35, 38) and not CEA.

Table 1  $x^2$ 2 × 2 contingency analysis of COL-4 reactivity with ovarian and colon carcinomas

<table>
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<th>Tissue Tumor type (%)</th>
<th>COL-4 &gt;1 cell</th>
<th>Statistical significance</th>
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<td>Primary colon adenocarcinomas vs. primary serous cystadenocarcinomas of the ovary</td>
<td>44.0</td>
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<td>All colon carcinomas vs. all ovarian carcinomas</td>
<td>115.7</td>
<td>$P &lt; 0.001$</td>
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<td>All colon adenocarcinomas vs. all mucinous and poorly differentiated ovarian carcinomas</td>
<td>44.1</td>
<td>$P &lt; 0.001$</td>
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<tr>
<td>Metastatic colon adenocarcinoma vs. primary mucinous and poorly differentiated ovarian carcinomas</td>
<td>11.45</td>
<td>$P &lt; 0.005$</td>
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<td>Metastatic colon adenocarcinomas vs. all primary ovarian carcinomas of the common epithelial histological types</td>
<td>35.1</td>
<td>$P &lt; 0.005$</td>
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<tr>
<td>Primary colon adenocarcinomas vs. all metastatic ovarian carcinomas of the common epithelial histological types</td>
<td>48.01</td>
<td>$P &lt; 0.001$</td>
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Table 2 Immunohistochemical reactivity of primary human tumors with MAb COL-4 and B1.1 using avidin-biotin-peroxidase complex immunohistochemical methods

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Cellular reactivity with MAb B1.1 (%)</th>
<th>Cellular reactivity with MAb COL-4 (%)</th>
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<tr>
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<td>60/50</td>
<td>60/50</td>
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<tr>
<td>Ovary</td>
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</tr>
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<td>Serous cystadenocarcinoma</td>
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<td></td>
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<tr>
<td>Mucinous cystadenocarcinoma</td>
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<tr>
<td></td>
<td>75/≤5</td>
<td>75/≤5</td>
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<tr>
<td></td>
<td>≤5/0</td>
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<tr>
<td>Breast</td>
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<td>Invasive ductal carcinoma</td>
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DISCUSSION

CEA, as originally described by Gold and Freedman (19), was defined as an immunoreactive glycoprotein found only in fetal and malignant gastrointestinal tissues. Subsequent studies, however, have demonstrated the antigenic heterogeneity of CEA epitopes defined by polyclonal and monoclonal antibodies, as well as the existence of CEA-related antigens present in normal human tissues (32–53). Hence, CEA has been further defined as a family of isoantigens with multiple antigenic determinants.

We have recently reported the generation of 15 anti-CEA MAbs (designated COL 1–15) which recognize at least five distinct and relatively restrictive epitopes of CEA (34). These MAbs were selected because of their minimal reactivity with a wide variety of normal human tissues (i.e., CEA-related antigens) and strong reactivity with adenocarcinomas of the colon. This study was undertaken to further define expression of the epitope recognized by MAB COL-4 in ovarian and gastrointestinal carcinomas, because ovarian tumors have been reported positive for CEA and our preliminary experiments demonstrated no reactivity with serous cystadenocarcinomas. Of particular interest was the application of MAB COL-4 as an immunohistochemical adjunct to the differential diagnosis of colon from ovarian carcinoma, because this is a difficult clinical and pathological distinction with serious therapeutic and prognostic implications. Our studies demonstrated (Table 2) that MAB COL-4, in contrast to anti-CEA MAB B1.1, was generally nonreactive with adenocarcinomas arising in the ovary or breast. Adenocarcinomas of the colon were generally reactive with COL-4, whereas common epithelial carcinomas of the ovary were not (P < 0.001). Metastatic colon carcinomas were also generally reactive with COL-4 when compared with primary mucinous or poorly differentiated ovarian carcinomas; hence, immunoreactivity strongly supports a diagnosis of metastatic colon cancer rather than a primary ovarian tumor (P < 0.005). It is important to note, however, that there is no absolute guarantee that those cases diagnosed as primary or metastatic mucinous tumors of the ovary or metastatic adenocarcinomas of the colon utilized in this study are in fact that. In each case the diagnosis was made using standard pathological criteria (see “Materials and Methods”), but because of the inherent nature of the disease processes themselves (which include frequent metastases of the colon to ovary and vice versa, as well as similarities in histological and biochemical parameters as discussed) absolute distinction is not possible.

Adenocarcinomas of the breast and stomach also commonly metastasize to the ovary, although the histological appearance of these tumors usually allows their differentiation from primary ovarian cancer. Generally, gastric carcinomas were strongly reactive with COL-4, and therefore may also be distinguished from primary ovarian tumors on the basis of COL-4 expression. Recent studies have shown that the vast majority of 40 gastric cancers are strongly reactive with MAB COL-4. Breast carcinomas, in contrast to gastric tumors, were generally nonreactive with COL-4.

Solid-phase radioimmunoassays and Western blots were utilized to quantitate MAB COL-4 and B1.1 immunoreactivity with ovarian and colon tumors. Radioimmunoassays confirmed the findings obtained by immunohistochemical analyses, i.e., MAB COL-4 was reactive with colon carcinomas and nonreactive with ovarian cancers. Colon carcinoma metastases to the ovary from two patients were also strongly reactive with COL-4. MAB B1.1, in contrast, was reactive with both ovarian (the mucinous type) and colon carcinomas. Western blotting experiments demonstrated that although both MAB COL-4 and B1.1 reacted with the M180,000 CEA protein in colon carcinomas, COL-4 was more selective and failed to recognize any epitope or other protein from the primary ovarian tumor extract. In addition, B1.1 recognized a lower molecular weight protein in the ovarian carcinoma but failed to react with a M180,000 band. This suggests that the epitope recognized by MAB B1.1 in ovarian tumors is distinct from the COL-4 antigen, and may in fact represent a cross-reacting antigen similar to that described previously (34).

* N. Ohuchi et al., submitted for publication.
Several studies have characterized other anti-CEA MAbs with selective reactivities to colon carcinoma versus normal tissues. DeBoer and Nayman (60) evaluated three intestine-associated antigens, including a small intestinal mucin, large intestinal mucin, and CEA using polyclonal antisera and immunohistochemical techniques, and found reactivity of all three with mucinous ovarian and colonic adenocarcinomas. From this observation it was concluded that (a) intestinal-type epithelium was present in cystic mucinous tumors of the ovary and (b) inappropriate intestine-associated mucin substances are secreted following malignant transformation. Catane et al. (61), using a monoclonal antibody, have shown that there is a difference in staining patterns between colorectal origin and breast cancer origin. In a recent study by Tohya et al. (62), CEA was purified from the cyst fluid of serous and mucinous ovarian tumors and evaluated biochemically utilizing polyacrylamide gel electrophoresis and liquid chromatography. Heteroantisera were generated against this purified CEA preparation, and utilized for immunological assays. Using this polyclonal antibody preparation (which most likely recognizes multiple epitopes on the CEA molecule), the reactivity in ovarian tumors closely resembled that in colon carcinomas. Our data, in contrast, suggests that although various epitopes of the CEA molecule are expressed by ovarian mucinous tumors, the epitope recognized by COL-4 was generally not detectable using immunohistochemical, radioimmunoassay, and Western blotting techniques. This knowledge may also be useful in those cases where separate primary tumors in the colon and ovary arise in a single patient; although this occurrence is rare, it has been reported within certain families and may have a distinct genetic inheritance pattern (63, 64). This syndrome, however, may not be recognized because the distinction of primary versus metastatic mucinous adenocarcinoma may be difficult using clinical and standard histological criteria.

The utilization of MAb COL-4 to assay body fluids such as sera or effusions may also have some value in distinguishing between colorectal and ovarian carcinoma. While the epitope recognized by COL-4 is expressed by only a low percentage of the malignant epithelial cells of rare mucinous tumors of probable ovarian origin, we believe the data presented here demonstrate that MAb COL-4 may be utilized to distinguish primary ovarian carcinomas from metastatic colon carcinomas and vice versa. Because the therapy and prognosis of colon and ovarian carcinomas are different, the ability to make this distinction is of tantamount clinical importance. Subsequent prospective studies utilizing antigenic phenotyping in addition to clinical and histological criteria for mucinous tumors resected from the abdomen will be required to demonstrate the practical utility of MAb COL-4 as an immunohistochemical adjunct for this important diagnostic differentiation.

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