Diffential Expression of Carcinoembryonic Antigen in Early Gastric Adenocarcinomas versus Benign Gastric Lesions Defined by Monoclonal Antibodies Reactive with Restricted Antigen Epitopes

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ABSTRACT

Murine monoclonal antibodies (MAbs) reactive with distinct epitopes on carcinoembryonic antigen (CEA) have been analyzed systematically by radiolabeled MAbs, immunohistochemical assays to define CEA expression in adenocarcinomas, benign lesions, and normal tissues of the stomach. Each of four COL-MAbs (COL-1, COL-4, COL-6, and COL-12) reacted preferentially with cell extracts of adenocarcinomas versus those of normal mucosa in solid-phase radioimmunoassays. Using Western blotting analyses MAbs COL-1, COL-4, COL-6, and COL-12 detected only the M, 180,000 molecule characteristic of CEA in adenocarcinoma of the stomach; no reactivity was observed in extracts of normal gastric mucosa. Antibody competition radioimmunoassays were then carried out to define reactions among COL-MAbs using 125I-radiolabeled MAbs, and nonradiolabeled MAbs as competitors. A spectrum of formalin-fixed, paraffin-embedded normal, benign, and malignant tissue sections of the stomach were examined for immunoreactivities with COL-MAbs using immunohistochemical assays to define whether the COL-MAbs were able to detect CEA expression in early foci of gastric carcinomas. All of the COL-MAbs generally demonstrated selective reactivities to adenocarcinomas (n = 40) versus benign lesions (n = 15) and normal mucosa (n = 6) of the stomach. From 72 to 100% of adenocarcinomas at early stage (n = 18) were reactive with the COL-MAbs, suggesting that these MAbs might serve as immunohistochemical diagnostic tools to detect early foci of gastric carcinomas. The data reported here indicate that the COL-MAbs can potentially be utilized as radiolimunological and immunohistochemical adjuncts to differentiate early adenocarcinomas from normal mucosa or benign lesions of the stomach on the basis of differential CEA expression.

INTRODUCTION

CEA² is among the most extensively studied oncofetal antigens. It has been described as a M, 180,000 complex glycoprotein which is highly expressed by both embryonic colonic mucosa and carcinomas of the gastrointestinal tract (1, 2). There are numerous studies, however, indicating that CEA may actually be a family of isoantigens rather than a single antigen, and that many anti-CEA antibodies indeed differ in their tissue reactivity (3, 4). Previous studies have shown that antibodies raised against CEA react with many different types of carcinomas as well as with antigenic determinants expressed in normal tissues (5-8). The CEA cross-reacting antigens most commonly described are nonspecific cross-reacting antigen (NCA) (5, 6), NCA-2 (5-7, 10), normal fecal antigen (8), meconium antigen (11), and biliary glycoprotein (12). Consequently, many MAbs reactive with CEA have been shown to react with CEA-related antigens in normal cells of various organs as well as in neoplastic tissues (13-20).

There are several areas in which MAbs to CEA are being used in the management of patients with gastrointestinal carcinomas. These include blood assays to monitor tumor burden, immunohistochemical analyses of tissue samples to detect or further characterize tumor cells, the in situ detection of carcinomas using radiolabeled antibody, and MAb-guided therapy.

Worldwide, the most common neoplasm is still cancer of the stomach, incidence rates of which are high in Europe, East Asia, and South America (21). This malignancy has a very poor prognosis, and mortality rates are extremely high, especially in Western countries (22, 23). Screening for stomach cancer by barium X-ray, fiberoptic endoscopy with biopsy and/or cytology, or sulfoglycoprotein antigen in gastric juice has been conducted in countries such as Japan and Finland. Nevertheless, gastric cancer is still the most common neoplasm in Japan, accounting for 49,000 deaths in 1983 (24). Histologically, it is difficult to detect early morphological change by carcinoma cells in tissue sections if the carcinoma cells are not obvious. Immunopathology has been performed using monoclonal antibodies on frozen and/or paraffin-embedded tissues of the stomach in which positive staining was ascribed to the presence of CEA (18, 25, 26). However, in the case of gastric cancer, a detailed characterization as to whether the epitopes recognized by these MAbs were restricted to the M, 180,000 CEA or shared by the CEA-related antigens has not yet been carried out. Furthermore, studies using MAbs with restricted CEA reactivity have not been undertaken to define whether early gastric adenocarcinomas and/or benign lesions express distinct epitopes of CEA.

We have recently described a panel of anti-CEA MAbs, designated COL-1 through COL-15, obtained from mice immunized with several different preparations derived from membrane-enriched fractions of human colon carcinoma biopsies (27). These MAbs were selected early in the screening process for lack of reactivity to polymorphonuclear leukocytes, the most common cross-reactive normal tissue of many anti-CEA MAbs. These MAbs have previously been shown to be preferentially reactive with carcinomas of the colon when compared to normal adult tissues, and to react only with the M, 180,000 CEA component and not lower molecular weight components in tumor extracts (27). In particular, MAbs COL-1, 4, 6, and 12 were selected for extensive investigation due to their ability to detect reactive epitopes in formalin-fixed, paraffin-embedded tissues. The present study was initiated to (a) detect levels of CEA expression in cell extracts of carcinomas and normal mucosa of the stomach using solid-phase radioimmunoassays, (b) detect CEA expression in stomach adenocarcinoma using Western blotting analysis, (c) better define whether the COL-MAbs recognize distinct epitopes on the CEA molecule using antibody competition radioimmunoassays, (d) investigate the potential utility of COL-MAbs as adjuncts to detect early foci of gastric carcinomas.
of carcinoma in a spectrum of benign and malignant gastric tissues using immunohistochemical techniques, and (e) define the parameters for the potential utility of selected COL-MAbs in MAb-guided in situ targeting of gastric carcinoma.

MATERIALS AND METHODS

Monoclonal Antibodies. MAbs COL-1, 4, 6, and 12 were generated using extracts or membrane-enriched fractions of biopsy material from either primary or metastatic colon carcinomas as sequential immunogens in BALB/c mice as previously described (27). Somatic cell hybrids were cloned twice by limiting dilution and hybridoma cell lines designated COL-1, 4, 6, and 12 were identified. Immunoglobulins of each MAb was purified from ascites fluid by ion-exchange chromatography (28) and used for solid-phase RIAs and immunohistochemical assays.

A single pool of hybridoma tissue culture supernatant of each COL-MAb was used for Western blotting analyses and immunohistochemical assays of frozen tissues. These COL-MAbs have been previously shown to be immunoreactive with high molecular weight glycoprotein complex, designated TAG-72 (29, 30). An isotype identical control MAb UPC-10 for IgG2a MAbs COL-1 and COL-4 and a control MAb MOPC-21 (31) for IgG1 MAbs COL-6, COL-12, and B72.3 were used as negative controls for all tissue samples.

Specimens. Three adenocarcinomas and their respective normal counterparts of the stomach were frozen in liquid nitrogen immediately after surgery. Furthermore, 3 specimens of normal stomach mucosa taken from patients with gastric ulcer were also snap-frozen in liquid nitrogen. Those frozen tissues were kept at -70°C either until they were cut into 5-µm sections for immunohistochemical assays or until they were homogenized to prepare cell extracts for radioimmunoassay or Western blotting analyses.

Formalin-fixed, paraffin-embedded tissues were obtained from 40 patients with gastric carcinoma, 15 with benign gastric lesions (6 with hyperplastic polyps, 5 with adenomatous polyps, and 4 with dysplastic lesions), and 6 patients with normal gastric tissues. All normal tissues were obtained from patients without a history of cancer. Gastric carcinomas were subdivided into 4 groups based on their histological patterns: well-differentiated tubular adenocarcinomas (n = 11), moderately differentiated tubular adenocarcinomas (n = 10), poorly differentiated adenocarcinomas (n = 12), and signet ring cell carcinomas (n = 9). Dysplastic lesions were diagnosed on hematoxylin- and eosin-stained sections based on the criteria proposed by Morson et al. (32). Gastric carcinomas involving the mucosa and/or submucosa were considered early carcinomas, while more invasive carcinomas found in the muscularis propria, subserosa, or serosa were classified as advanced carcinomas (33, 34). Five-µm sections of paraffin-embedded tissues were cut and mounted on gelatin-coated glass slides. Tissue sections from each specimen were stained with hematoxylin and eosin for cellular visualization.

Cell Extract Preparation. Tissues were homogenized for 3 min on ice in 10 mM Tris-HCl (pH 7.2) with 0.2 mM CaCl2 (10 g/100 ml). The homogenate was subjected to pressure homogenization using a cell disruptor bomb (Parr Instrument Co., Moline, IL) for 10 min at 1000 lb/in2 and clarified at 2800 × g for 5 min. The supernatant was sonicated on ice for 2 min with 15-s intervals (Branson sonifier). The sonicate was then centrifuged at 9500 × g for 10 min. The supernatant was used in solid-phase RIAs and Western blotting analyses. Protein concentration was determined by the method of Lowry et al. (35).

Solid-Phase Radioimmunoassays. Fifty µl containing 5 µg of cell extract from normal or malignant gastric tissues were added to each well of a 96-well polyvinyl microtiter plate and allowed to dry overnight at 37°C in a nonhumidified incubator. The wells were then treated to minimize nonspecific protein binding by the addition of 100 µl of 5% BSA in PBS with Ca2+ and Mg2+ for 1 h. The BSA was aspirated, and 50 µl containing serial dilutions of MAbs COL-1, 4, 6, and 12 (from 100 to 0.01 ng of IgG) were added to each well. After 1 h of incubation the unbound IgG was removed, and the plates were washed with 1% BSA in PBS. Each well was then incubated with 125I-labeled goat-anti-mouse IgG (Kirkegard and Perry, Gaithersburg, MD) at 75,000 cpm in 25 µl for 1 h at 37°C. The unbound 125I-goat-anti-mouse IgG was aspirated, and the wells were washed with 1% BSA in PBS. The bound 125I-goat-anti-mouse IgG was detected by cutting the individual wells and measuring the radioactivity in a gamma counter.

Western Blotting Analyses. Forty µg of each cell extract were diluted in SDS-polyacrylamide gel electrophoresis sample buffer (0.125 M Tris-HCl, pH 6.8; 4% SDS; 20% glycerol; 10% 2-mercaptoethanol), and loaded onto a 5 to 20% linear gradient SDS-polyacrylamide gel electrophoresis. 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 MAbs COL-1, 4, 6, and 12 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-labeled goat-anti-mouse IgG (Kirkegard and Perry) for 1 h. 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 3 h. For each experiment the tissue culture supernatant of parent mouse myeloma cell line (NS-1) was used as a negative control.

Competition Solid-Phase Radioimmunoassays. To determine if each COL-MAb recognized a distinct antigenic determinant of CEA molecule, competitive binding assays were performed. MAbs COL-1, 4, 6, 12, and B72.3 were tested for their ability to compete for the binding of 125I-labeled MAbs COL-4 and COL-12 to an extract of gastric carcinoma cells. Purified immunoglobulin of MAbs COL-4 and 12 was iodinated following the iodogen procedure (3). Five µg of gastric carcinoma cell extract were adsorbed to each well of polyvinyl microtiter plates, and a saturating amount of first (inhibitor) MAb (from 10 to 0.004 µg/50 µl) was added. After incubation for 6 h at 4°C, either 125I-labeled MAb COL-4 or 125I-labeled MAb COL-12 (500,000 cpm/25 µl) was added to each well and incubated for 12 h at 4°C. Bound 125I-labeled COL-MAb was determined by cutting individual wells and measuring radioactivity in a gamma counter. Cpm bound to the wells preincubated with saturating MAbs COL-1, 4, 6, 12, or B72.3 as competitors in their respective assays was considered 100% competition.

Immunohistochemical Method for Frozen Tissues. Five-µm sections of frozen tissues were incubated in methanol for 5 min, then treated in methanol containing 0.3% H2O2 for 10 min to block endogenous peroxidase activity. After washing in PBS, the sections were pretreated with 10% normal horse serum. Serial sections were then incubated with undiluted tissue culture supernatants of primary MAbs COL-1, 4, 6, and 12 for 30 min. An isotype identical control MAb UPC-10 or MOPC-21 was also added. After the incubation with primary MAbs, the sections were processed by the same method as described below for formalin-fixed, paraffin-embedded tissue sections.

Immunohistochemical Methods for Formalin-fixed, Paraffin-embedded Tissues. Formalin-fixed, paraffin-embedded human gastric tissues were investigated for CEA expression using ABC immunohistochemical methods. Briefly, 5-µm sections were cut and mounted on gelatin-coated slides. The sections were deparaffinized in xylene and rehydrated in graded alcohols. Endogenous peroxidase activity was blocked using methanol containing 0.3% H2O2 for 10 min. After rinsing in PBS, the sections were pretreated in 10% normal horse serum for 15 min. This and all subsequent reagents were diluted in PBS with 0.1% bovine serum albumin. The pretreatment serum was removed and purified primary MAbs COL-1, 4, 6, and 12 were added on serial sections at appropriate concentration of each MAb based on titration assays using normal and malignant gastric tissues (COL-1 and 4 at 20 and COL-6 and 12 at 40 µg/ml) for 30 min at room temperature. An isotype identical control MAb UPC-10 or MOPC-21 was also added on serial sections as negative control for these MAbs. After a rinse with...
RESULTS

Solid-Phase Radioimmunoassays. MAbs COL-1, 4, 6, and 12 were characterized as to their reactivities to adenocarcinomas and normal mucosae of the stomach using solid-phase RIAs. Cell extracts from 3 primary adenocarcinomas, their respective adjacent normal mucosae, and 3 normal mucosae from patients with gastric ulcer were assayed with each of the purified IgG preparations of the 4 COL-MAbs. As shown in Fig. 1, all 4 COL-MAbs reacted preferentially with all 3 stomach adenocarcinomas, whereas they were either weakly reactive or not reactive with normal mucosae from patients with gastric adenocarcinoma. MAbs COL-1 and 4 demonstrated higher reactivity with adenocarcinomas when compared to MAbs COL-6 and 12 at the same immunoglobulin inputs. However, these 2 MAbs (COL-1 and 4) demonstrated weak reactivity with normal mucosae from patients with carcinoma, whereas MAbs COL-6 and 12 were essentially nonreactive with these normal mucosae. Tissue extracts of normal mucosa from 3 patients with gastric ulcer were also examined, and the average cpm bound obtained from the 3 specimens was shown in Fig. 1 (●). Less than 1000 cpm bound was observed in each assay at the highest concentration of COL-MAbs (100 ng) used, demonstrating that COL-MAbs were less reactive with normal mucosae from patients without carcinoma than those from patients bearing carcinoma.

Western Blotting. MAbs COL-1, 4, 6, and 12 were then characterized as to their ability to detect CEA in adenocarcinoma of the stomach using Western blotting analysis. As seen in Fig. 2, all of the COL-MAbs immunodetected the Mf, 180,000 protein characteristic of the CEA molecule in the carcinoma cell extract but not in the extract from normal stomach mucosa. Note the absence of the lower molecular weight components (30,000 to 90,000) seen with the many anti-CEA MAbs (16, 19, 20).

Competition Solid-Phase Radioimmunoassays. To further define whether COL-MAbs recognize different antigenic determinants on the CEA molecules, competitive solid-phase RIAs were performed. Nonradiolabeled MAbs were used as competitors for each experiment. Purified MAbs COL-4 and 12 were radiolabeled with $^{125}$I, and each was bound in limiting dilution to a cell extract from gastric adenocarcinoma. Various amounts of unlabeled purified IgGs of MAbs COL-1, 4, 6, 12, and B72.3 were then added as competitors in each assay. As seen in Fig. 3A, unlabeled IgG MAb COL-4 competed completely with $^{125}$I-labeled MAb COL-4. MAbs, COL-1 and 6 competed in this

![Fig. 1. Reactivities of MAbs COL-1, 4, 6, and 12 to extracts from adenocarcinomas and normal mucosae of the stomach in solid-phase RIAs. Five µg of each extract were reacted with each MAb as described in "Materials and Methods." M, A, extracts of adenocarcinomas; O, D, extracts of normal adenocarcinomas; D, O, extracts of normal mucosae from patients with gastric carcinoma. Each D, O, an extract of normal tissue adjacent to the gastric carcinoma shown by the same M, A, O, extracts of normal mucosae from patients with gastric ulcer (an average cpm bound obtained from 3 normal mucosae).](3567)

![Fig. 2. Immunodetection of CEA by MAbs COL-1 (Lanes A and B), COL-4 (Lanes C and D), COL-6 (Lanes E and F), COL-12 (Lanes G and H), and negative control NS-1 (Lanes I and J) using Western blotting analyses. Immunoblotting was performed as described in "Materials and Methods." Each lane represents one of the 4 COL-MAbs immunodetected the $A/r$ 180,000 protein characteristic of the CEA molecule in the carcinoma cell extract but not in the extract from normal stomach mucosa.](3567)

![Fig. 3. Competition solid-phase RIAs using $^{125}$I-labeled MAb COL-4 (A) and $^{125}$I-labeled MAb COL-12 (B) as described in "Materials and Methods." Different amounts of purified MAbs COL-1 (O), COL-4 (●), COL-6 (□), COL-12 (△), and B72.3 (△) were used as competitors. A, MAb COL-4 competed completely. In contrast, MAbs COL-12 and B72.3 did not compete. B, MAb COL-12 competed completely with $^{125}$I-labeled MAb COL-12, whereas MAbs COL-1, 4, 6, and B72.3 did not.](3567)
assay but not with the efficiency of MAb COL-4. In contrast, MABs COL-12 and B72.3 (which reacts with TAG-72) did not compete with $^{125}$I-labeled MAb COL-4. As shown in Fig. 3B, MAB COL-12 competed completely with $^{125}$I-labeled MAB COL-12, while MABs COL-1, 4, 6, and B72.3 did not compete with $^{125}$I-labeled MAB COL-12. Competition studies performed using $^{125}$I-labeled COL-4 and 6 demonstrated similar results with nearly complete competition by COL-1, 4, and 6 Mabs with no inhibition by COL-12. These studies demonstrate that MAB COL-12 recognizes an epitope on the CEA molecule which is distinct from epitopes recognized by MABs COL-1, 4, and 6. While MABs COL-1, 4, and 6 do cross-inhibit each other’s binding in these competition assays, previous direct (27) and immunohistochemical binding studies (see below) demonstrated different binding patterns for each of these 3 COL-MAbs. These Mabs must bind to epitopes that are sterically close to each other. These experiments also demonstrated that the COL-MAbs recognized CE A determinants distinct from TAG-72 antigen.

**Immunoreactivity of COL-MAbs with Frozen Gastric Tissues.** Frozen sections of 2 adenocarcinomas and their respective normal counterparts of the stomach were tested with MABs COL-1, 4, 6, and 12 using ABC immunohistochemical method (Fig. 4). The adenocarcinomas of the stomach reacted with each of the COL-MAbs, with ≥80% of carcinoma cells positive at 1:1 and 1:4 dilutions of tissue culture supernatant of MAB, and ≥45% at 1:16 and 1:64 dilutions. As can be seen in Fig. 4, a strong differential reactivity for gastric carcinoma versus normal gastric epithelium was observed at each MAB dilution with each of the COL-MAbs. The apical surface of the superficial epithelial cells of normal mucosa was occasionally reactive with each of COL-MAbs.

**Immunoreactivity of COL-MAbs with Formalin-fixed Gastric Tissues.** A spectrum of formalin-fixed, paraffin-embedded normal, benign, and malignant stomach tissues was analyzed using ABC immunohistochemical techniques to determine whether the COL-MAbs were reactive with formalin-fixed gastric tissues and to examine the reactivity of each MAB to carcinoma cells versus benign or normal gastric epithelium. As shown in Fig. 5, each of the COL-MAbs demonstrated preferential reactivity to adenocarcinomas versus normal mucosa and benign lesions of the stomach. MAB COL-1 reacted with 35 of 40 (88%) adenocarcinomas, 6 of 14 (43%) benign lesions, and 2 of 6 normal mucosa. However, 22 of 40 (55%) carcinomas demonstrated strong reactivity with ≥20% of cell positive, while only 2 benign lesions (one dysplastic lesion and one hyperplastic polyp) and none of normal mucosa showed similar reactivity (Fig. 5, A and B). MAB COL-4 reacted with all of the adenocarcinomas tested. Thirty-two of 40 (80%) adenocarcinomas demonstrated that ≥20% of carcinoma cells with MAB COL-4, were reactive, whereas only 2 benign lesions were similarly reactive (Fig. 5, C and D).

MABs COL-6 and 12 showed slightly lower average percentages of reactivity to adenocarcinomas, as compared to MABs COL-1 and 4. MAB COL-6 reacted with 33 of 40 (83%), and MAB COL-12 was reactive with 32 of 40 (80%) adenocarcinomas of the stomach. None of the benign lesions demonstrated ≥10% of cells positive with either MAB COL-6 or 12, and all of the normal mucosa were completely negative (Fig. 5, E to H).

Fig. 6 illustrates representative immunohistochemical stainings of COL-MAbs with serial sections of the same tissues, demonstrating that the different COL-MAbs can react with distinct carcinoma cells. For example, COL-12 reacts more strongly with a poorly differentiated stomach adenocarcinoma while a serial section stained with COL-6 shows less staining (Fig. 6, A and B). Conversely, using the identical purified Mab IgG preparation, serial sections of a signet ring carcinoma show more intense staining with MAB COL-6 than with COL-12 (Fig. 6, C and D). Another example of this type of differential reactivity is seen in Fig. 6, E and F which compare the reactivity of COL-4 and 6, respectively, with a well-differentiated gastric adenocarcinoma.

As seen in Fig. 5, among the benign lesions examined, 3 of 4 dysplastic lesions were reactive in epithelial cells with atypia, abnormal differentiation, and disorganized mucosal architecture with MABs COL-1, 4, and 12. Two of 4 dysplastic lesions reacted with MAB COL-6.

Among the 15 benign lesions, 12 showed intestinal metaplasia, determined by staining a serial section with Alcian blue at pH 2.5. Three of the COL-MAbs, COL-1, 6, and 12 were completely negative when reacted with intestinal metaplasia of gastric mucosa.

**Detection of Early Gastric Carcinoma by COL-MAbs.** To further determine whether the COL-MAbs were able to detect early foci of adenocarcinoma cells of the stomach, and whether MAB reactivity correlated with tumor invasion, immunohistochemical reactivities were defined in relation to depth of tumor invasion (Table 1). All of the COL-MAbs reacted with all carcinomas which had invaded into subserosa and serosa. At least 7 of 10 carcinomas confined within mucosa (the earliest stage of carcinoma) were reactive with each of COL-MAbs with MAB COL-4 reacting with 10 of 10 early gastric carcinomas.
Fig. 6. Differential reactivities of COL-MAbs to gastric adenocarcinoma using ABC immunoperoxidase techniques on serial tissue sections. COL-12 (A) and 6 (B) reacted with a poorly differentiated gastric adenocarcinoma; COL-6 (C) and 12 (D) reacted with a signet ring adenocarcinoma; and COL-4 (E) and 6 (F) reacted with a well-differentiated tubular adenocarcinoma. Note the differential reactivity of each of the COL-MAbs to carcinoma cells and the lack of staining of normal gastric gland (arrowheads). Counterstained with hematoxylin; original magnifications, ×220.

| Table 1 Reactivity of MAbs COL-1, -4, -6, and -12 with gastric adenocarcinomas in relation to depth of tumor invasion |
|--------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Depth of tumor invasion              | COL-1 | COL-4 | COL-6 | COL-12 |
| Mucosa                               | 9/10  | 10/10 | 7/10  | 8/10  |
| Submucosa                            | 6/8   | 8/8   | 6/8   | 6/8   |
| Muscularis propria                   | 7/9   | 9/9   | 7/9   | 5/9   |
| Subserosa                            | 7/7   | 7/7   | 7/7   | 7/7   |
| Serosa                               | 6/6   | 6/6   | 6/6   | 6/6   |
| Early carcinomas*                    | 15/18 (83)* | 18/18 (100) | 13/18 (72) | 14/18 (78) |
| Advanced carcinomas*                 | 20/22 (91)* | 22/22 (100) | 20/22 (91) | 18/22 (82) |

* Early gastric carcinomas involving only the mucosa and/or submucosa.

DISCUSSION

Four monoclonal antibodies reactive with CEA have been utilized in RIAs and immunohistochemical assays to define CEA expression in adenocarcinomas, benign lesions, and/or normal mucosae of human stomach tissues. Western blotting analyses clearly demonstrated a capability of MAbs COL-1, 4, 6, and 12 to detect only the M, 180,000 protein characteristic of the CEA molecule in adenocarcinoma of the stomach, but not in normal gastric mucosa. Competitive RIAs using 125I-labeled MAbs demonstrated that MAB COL-12 recognized a distinct epitope on the CEA molecule from epitopes which were recognized by MAbs COL-1, 4, and 6. These assays also demonstrated that MAbs COL-1, 4, and 6 were similar and distinctive from MAB COL-12. However, the direct binding studies reported here and the studies reported previously (27) demonstrated each of MAbs COL-1, 4, and 6 to have distinct binding patterns. One possible explanation of these results is that the epitopes recognized by MAbs COL-1, 4, and 6 are close to one another on the CEA molecule, and thus these MAbs demonstrate steric inhibition in competition assays.

Studies of epitope mapping on CEA have been described by several investigators (36–38). Bosslet et al. (36) reported 3...
murine MAbs reactive to different epitopes on CEA, and Har-wood et al. (37) described 6 MAbs which were specific for CEA and reacted with at least 3 unrelated regions of the CEA glycoprotein. We have previously demonstrated 15 COL-MAbs reactive with at least 5 distinct epitopes on the CEA molecule (27) by means of surface binding to colon carcinoma cell lines using fluorescence-activated cell sorting, and differential binding to breast and bladder carcinoma or rhabdomyosarcoma cell extract using solid-phase RIAs.

Solid-phase RIAs showed that MAbs COL-1, 4, 6, and 12 were strongly reactive with cell extracts of adenocarcinomas, while these MAbs were either very weakly or not reactive with cell extracts of normal mucosa, suggesting that these MAbs may be useful for the radioimmunodetection of adenocarcinomas of the stomach. It is interesting to note that MAbs COL-1 and 6 were more reactive with normal mucosa from patients with carcinoma than those from patients without carcinoma, although the levels of CEA expression were quite lower than those of their respective carcinoma lesions.

The immunohistochemical studies reported here provide an evaluation of individual MAb reactivity to a wide range of gastric lesions at the single cell level. MAbs COL-1, 4, 6, and 12 demonstrated clear preferential reactivity to adenocarcinoma cells versus benign and/or normal gastric epithelial cells; moreover, they did not react with polymorphonuclear leukocytes or monocytes, a feature characteristic of many anti-CEA MAbs (16, 19, 20). It should be noted that 3 or 4 dysplastic lesions were reactive with MAbs COL-1, 4, and 12, since dysplastic lesions of the stomach have been associated with gastric adenocarcinoma (32, 39). The cellular reactivities of the COL-MAbs with adenocarcinomas were evaluated and compared to depth of carcinoma invasion. While the more invasive carcinomas, e.g., carcinomas invading into the subserosa, or serosa, demonstrated slightly higher reactivities than early carcinomas, 72 to 100% of carcinomas at early stage (carcinoma cells confined to only the mucosa and/or submucosa) were reactive with the COL-MAbs. Among the early gastric carcinomas tested, all of the signet ring cell carcinomas and poorly differentiated adenocarcinomas were positive with the COL-MAbs. This finding may provide the means, using the COL-MAbs, to detect carcinoma cells of the very aggressive type at their early stage. It is often very difficult to diagnose these dissociating types of carcinoma, i.e., signet ring cell carcinoma and poorly differentiated adenocarcinoma, and the patients with these types of carcinoma have a worse prognosis than the patients with associating types of carcinoma, i.e., well and moderately differentiated tubular adenocarcinoma (40). Using >20% cellular reactivity as a cutoff point, only 2 benign lesions reacted with 2 of the 4 COL-MAbs, whereas at least 55% of the adenocarcinomas showed that amount of cellular reactivity; the difference may be clinically useful. To define this, however, a large number of benign tumors (with appropriate patient follow-up) must be assayed.

We have recently investigated the expression of tumor-associated glycoprotein (TAG-72) in benign and malignant lesions of the stomach using MAb B72.3 and found that the vast majority of adenocarcinomas and premalignant lesions express the TAG-72 antigen (41). TAG-72, which has been characterized as a mucin-like molecule with $M_r > 10^6$ (42), has properties quite distinct from that of CEA. This is consistent with the data obtained here by competition RIAs (Fig. 3) showing that MAb B72.3 did not compete in the COL-MAb assays. Furthermore, when MAbs B72.3 and COL-6 were simultaneously reacted with the same carcinoma tissues, more carcinomas were detected as compared to the number of carcinomas shown to be reactive with the individual MAb (41, 43). Thus, in light of the degree of antigenic heterogeneity observed in most human carcinomas, the use of cocktails of MAbs reactive with different antigens, e.g., COL-MAbs (which react with CEA) and MAb B72.3 (which reacts with TAG-72), and/or MAbs reactive with distinct epitopes on the same antigenic molecule, e.g., COL-4 and 12, may be essential for the application of MAbs in radioimmunological and/or immunohistochemical adjuncts to detect carcinomas of the stomach.

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