Histol Histopath (1990) 5: 199-204

Histology and Histopathology

Distribution of the carcinoembryonic antigen CEA in gastric lesions. Immunohistochemical testing of three novel monoclonal antibodies

Aasmund Berner[1], Ole Børmer[2], Per F. Marton[1,3] and Jahn M. Nesland[1,3]

Departments of [1]Pathology and [2]Clinical Chemistry, The Norwegian Radium Hospital and [3]Institute for Cancer Research, Oslo, Norway

Summary. Three mouse monoclonal antibodies MAB (CEA 12-140-1, -2 and -4) raised against different CEA epitopes were tested in 32 gastric adenocarcinomas (18 intestinal type and 14 diffuse type) and 34 gastric lesions with severe and moderate dysplasia. The MAB stained 13, 11 and 13 out of the 14 diffuse carcinomas and 11, 13 and 13 out of the 18 intestinal carcinomas. The dysplastic lesions were positive in 9, 9 and 6 out of 34 cases. Less than half of the cases with metaplastic epithelium adjacent to the carcinomas were also positive for MAB. All MAB showed the same pattern of reactivity without cross-reactivity. Their cumulative staining rate corresponded closely to that of polyclonal CEA antiserum, but the MAB stained more cells. The reactivity was confined to intracytoplasmic vacuoles in diffuse carcinomas and appeared diffusely in the cytoplasm or limited to the cell membrane in intestinal type of carcinomas. Our findings do not indicate CEA to be a reliable marker for malignant transformation in gastric mucosa.

Key words: Carcinoembryonic antigen, Monoclonal antibodies, Immunocytochemistry, Gastric mucosa

Introduction

Carcinoembryonic antigen (CEA) was first described by Gold and Friedman and is now widely used as a tumour marker (Winawer, 1979). The fact that CEA shares antigenic epitopes with a group of other glycoproteins may give rise to unspecific reactions in immuno-stainings (Haggarty et al., 1986) which reduce the diagnostic value of polycolonal antisera raised against CEA. We have compared the specificity of 3 novel monoclonal antibodies (MAB) with that of a commercially available polyclonal CEA antiserum by testing their action on normal gastric mucosa and on various gastric lesions.

Offprint requests to: Dr. Aasmund Berner, Department of Pathology, The Norwegian Radium Hospital, Montebello 0310, Oslo 3, Norway

Materials and methods

32 resection specimens of gastric carcinoma and 30 gastroscopic biopsies from lesions showing moderate and severe dysplasia (15 of each) were taken from our files. All specimens were fixed in buffered 10% formalin and embedded in paraffin. Five μm sections were stained with haematoxylin and eosin for light microscopy. The carcinomas were classified according to Lauren (1965) and the dysplastic lesions were graded as recommended by WHO (Morson et al., 1980).

Monoclonal antibodies

Mouse hybridomas were produced by using purified CEA as antigen (Børmer, 1990). After an initial characterization of antibody affinity and specificity, 6 potentially useful clones were selected. They were repeatedly subcloned and expanded as ascites tumours. The monoclonal antibodies, denoted 12-140-1, -2, -4, -5, -7 and -10, were then purified from ascites fluid by Protein A-Sepharose Chromatography. Their dissociation constants were estimated from the binding of 125 J-CEA. The epitope specificities of all the antibodies had been mapped by cross inhibition experiments in an international workshop (Hammarstrøm et al., 1990) except antibody 12-140-2 which was evaluated in our laboratory. In short, antibodies 12-140-5, -7 and -10 reacted with the same epitope group (GOLD 4), whereas antibodies 12-140-1, -2 and -4 recognized different epitopes (GOLD 5, 2 and 1, respectively). The antibodies were further characterized in an immunohistological set-up with stained sections from spleen, liver, large intestinal mucosa and lung tissue. We selected 3 monoclonal antibodies without apparent cross-reactivity (CEA 12-140-1, -2 and -4) from the results (Table 1) for further study. The optimal staining conditions were found in the same way (Table 2).

Immunohistochemistry

Sections which had been fixed in formalin and embedded in paraffin were stained by the avidin-

biotin-peroxidase complex (ABC) method (Hsu et al., 1981) using the 3 monoclonal antibodies and a polyclonal CEA antiserum (Dakopatts) which was preabsorbed in splenic tissue. After removal of paraffin, the sections were treated for 30 minutes with 0.3% hydrogen peroxide in methanol to block endogenous peroxidase before incubation for 20 minutes with normal serum diluted 1:75 in 0.01 M saline (PBS), pH 7.4, containing 5% bovine serum albumin (BSA) to eliminate non-specific staining. They were then incubated at 4°C with the antibodies followed by a 30-minute incubation with a 1:200 dilution of the biotin-labelled second layer antibody and a 60- minute incubation with ABC (10 µg/ml avidin and 2.5 µg/ml biotin-labelled peroxidase) (Vector, Burlingame, CA). After further incubation for 5 minutes in freshly prepared 0.05% 3.3' diaminobenzidine-tetra-hydrochloride in 0.05 M Tris buffer, pH 7.6 containing 0.01% H_20_2, the sections were counterstained with haematoxylin, dehydrated and mounted.

Control studies included: 1) relevant positive control sections and 2) the use of non-immune serum of IgG fractions as first layer. The immunostained sections were examined independently by two pathologists. Localization of the staining product was noted and the number of positive cells evaluated semi-quantitatively as follows:

No positive cells:	0
Less than 1/3 of cells positive:	+
1/3 - 2/3 of cells positive:	++
More than 2/3 of cells positive:	+++

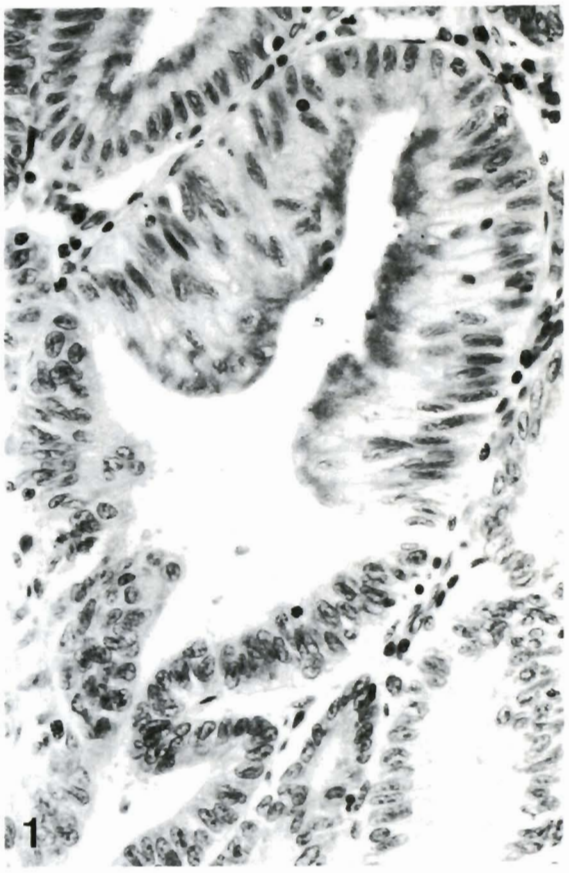

Fig. 1. Well-differentiated adenocarcinoma of intestinal type. Immunostaining with monoclonal antibody CEA 12-140-4. CEA reactivity is present in the apical part of the cytoplasm and along the cell membrane. × 400

Results

Light microscopy

Gastric carcinomas: 18 of the gastric carcinomas were of intestinal type and showed tubular, acinar and/or papillary growth patterns, often with varying patterns in different areas of the same tumour. Fourteen were diffusely infiltrating consisting of poorly cohesive cells with eccentric nuclei and varying amounts of intracytoplasmic mucin.

Gastric mucosa adjacent to the carcinoma: Adjacent gastric mucosa was included in the sections from 22 of the 32 carcinomas. Eleven showed intestinal metaplasia without atypia, while 7 showed normal mucosa. Epithelial dysplasia with intestinal metaplasia was found in 4 cases, 2 severe and 2 moderate, and occurred only in mucosa with intestinal metaplasia.

Immunohistochemistry

General observations. All the positive cells were patchily distributed on a clear background. More cells tended to be stained with the MAB than with the polyclonal anti-CEA. All cells positive for one or more MAB were also stained by the polyclinal antibody. No difference between the MAB was observed in the staining pattern.

A: Gastric carcinomas

Immunoreactivity with the polyclonal anti-CEA was found in 15 out of 18 carcinomas of the intestinal type, while the MAB were positive in 11, 13 and 13 out of 18 tumours (Table 3). The cumulative staining rate for the MAB was 15 out of 18. Three tumours were negative for all antibodies applied. The reactivity appeared either diffusely in the cytoplasm of the tumour cells (Fig. 1) or was limited to the cell membrane (Fig. 2). The polyclonal anti-CEA reacted with the tumour cells in 13 out of 14 diffuse carcinomas, while the MAB showed CEA reactivity in 13, 11 and 13 out of 14 tumours (Table 4). The cumulative detection rate for the MAB was 13 out of 14. One tumour was negative for all antibodies applied. The immunoreactivity of the tumour cells was confined to aggregates of cytoplasmic vacuoles (Fig. 3).

B: Gastric epithelium adjacent to tumours

No reactivity was found in normal gastric epithelium, except for one case where an area of cardiac mucosa

Table 1. Immunoreactivity of six MAB with formalin-fixed, paraffin-embedded normal tissue.

Antibody	spleen	liver	lung	colon mucosa*
12-140-1	–	–	–	+++
12-140-2	–	–	–	+++
12-140-4	–	–	–	+++
12-140-5	+++	++	+++	++
12-140-7	+++	+	+	+
12-140-10	+++	++	–	++

* Immunostaining of the apical part of surface mucosal cells.

Table 2. Staining conditions.

antibody	code	incubation	dilution
MAB 1	CEA-12-140-1	22 hours	1/1000 (1μg/ml)
MAB 2	CEA-12-140-2	22 hours	1/1000 (1μg/ml)
MAB 3	CEA-12-140-4	22 hours	1/1000 (1μg/ml)
Polycl. anti CEA	(Dakopatts)	22 hours	1/1000 (1μg/ml)

Table 3. Cea reactivity in intestinal type of carcinoma.

Compartment	Intensity	Polyclonal anti-CEA	MAB1	MAB2	MAB3
cytoplasm	+	2	2	0	2
	++	8	3	5	4
	+++	0	3	5	4
cell-membrane	0	3	7	5	5
	+	4	1	1	1
	++	1	1	2	2
	+++	0	1	0	0
total pos.		15	11	13	13

Table 4. CEA reactivity in diffuse carcinomas.

Compartment	Intensity	Polyclonal anti-CEA	MAB1	MAB2	MAB3
cytoplasmic vacuoles	0	1	1	3	1
	+	5	2	4	1
	++	7	6	4	6
	+++	1	5	3	6
total pos.		13	13	11	13

Table 5. CEA reactivity in metaplastic epithelium adjacent to carcinomas.

Compartment	Intensity	Polyclonal anti-CEA	MAB1	MAB2	MAB3
luminal surface	0	8	10	11	11
	+	7	5	4	5
	++/+++	0	0	0	0
total pos.		7	5	4	5

Table 6. CEA reactivity in moderate and severe dysplasia.

Compartment	Intensity	Polyclonal anti-CEA	MAB1	MAB2	MAB3
cytoplasm	+	0	1	1	0
luminal surface	0	23	25	25	28
	+	10	5	5	4
	++	1	3	3	2
total pos.		11	9	9	6

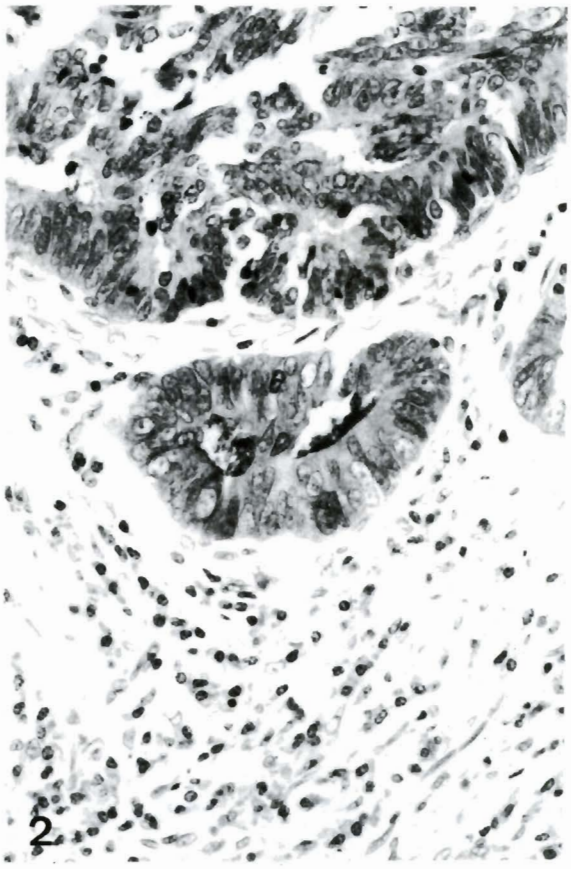

Fig. 2. Carcinoma of intestinal type. Immunostaining with monoclonal antibody CEA 12-140-1. A strong immunoreactivity is found diffusely in the cytoplasm and along the cell membrane. × 400

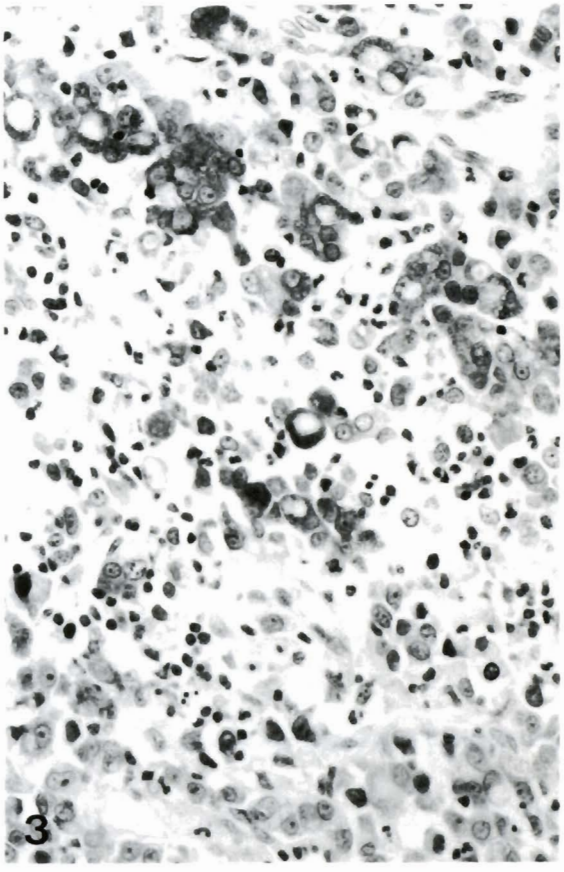

Fig. 3. Diffuse type of carcinoma. Immunostaining with monoclonal antibody CEA 12-140-2. Tumour cells with a stron intracytoplasmic immunoreactivity. × 400

showed positive surface staining with polyclonal anti-CEA and MAB 2. In 11 cases with non-dysplastic intestinal metaplasia immunoreactivity with the polyclonal anti CEA was found in 7 and the MAB were positive in about half of the cases with a cumulative detection rate of 5 out of 11 (Table 5). When positive, the CEA reactivity was limited to the luminal part of the cell membrane.

C: Gastric epithelial dysplasia

Two cases with moderate and two with severe dys plasia were included in the study. The polyclonal CEA was positive in 11 out of 34, while the cumulative detection rate for the MAB was 10 out of 34 (Table 6). Except for one case with positive staining of cytoplasmic vacuoles (MAB 1 and 2) the CEA reactivity was always confined to the luminal part of the cell membrane.

Discussion

In epithelium from the various gastric lesions the MAB showed closely corresponding results regarding reactivity, number of positive cells and staining intensity. MAB 2 and 3 scored highest (13 out of 18) in carcinomas of intestinal type, while MAB 1 and 3 scored highest in

the diffuse type (13 out of 14). The difference between highest and lowest score was 2 out of 18 and 2 out of 14. The correspondence between MAB was even better in metaplastic and dysplastic epithelium. The cumulative staining rate of the MAB in the carcinomas was identical to the immunoreactivity of the polyclonal anti-CEA. The cumulative staining rate was slightly lower in metaplastic and dysplastic epithelium.

A polyclonal antiserum contains a number of specific antibodies which react with different epitopes on the antigen. Some of these antibodies may give rise to cross-reactivity with related antigens and this reduces the specificity of the antiserum. In order to minimize this, polyclonal anti-CEA antibodies must be preabsorbed with a relevant tissue powder. However, some cross-reactivity may still remain. We found somewhat broader immunoreactivity in non-malignant lesions with the polyclonal antibody than with our MAB. When comparing the results it is difficult to decide whether the reduced reactivity is due to the low sensitivity of the MAB or to a false positive reaction due to cross-reactivity of the polyclonal antibody.

The cumulative immunoreactivity of the three MAB corresponded closely to that of the polyclonal antibody and no cross-reactivity was observed. In our opinion the possible disadvantage of reduced sensitivity of the MAB is compensated by a better quality of the stain and by avoidance of preabsorption of the antibody. The problem of reduced sensitivity could be overcome in future by use of a broader panel of MAB. A mixture of several monoclonal antibodies would avoid the inconvenience of staining several sections.

Gastric carcinomas are histologically heterogeneous and their varying CEA expression is probably a biochemical reflection of this (Hockey et al., 1984). Like Ohuchi et al. (1987) we observed the highest frequency of immunoreactivity in diffuse carcinomas. It is in this type of carcinoma that staining for CEA may serve to identify small groups of tumour cells that might otherwise be overlooked.

CEA distribution in the subcellular compartments of the tumour cells was investigated by Lindgren et al. (1979) who did not find any correlation with the histological type. Nielsen et al. (1982) reported the reactivity to be intracytoplasmic in the diffuse type, and limited to the cell membrane and/or the apical cytoplasm in the intestinal type of carcinoma. Our results are in agreement with theirs.

We also confirmed the finding of Borch (1987) and Stramignoni (1985) who observed a significantly lower CEA reactivity in dysplastic and metaplastic epithelium than in carcinomas. When evaluating the real frequency of CEA expression, however, due reservation should be made on account of the small size of the biopsies. The patchy distribution of the immunoreactivity should also be borne in mind. Wurster et al. (1979) judged CEA to be a reliable marker for malignant transformation in gastric epithelium. Although we found a higher reactivity in carcinomas than in metaplastic and dysplastic epithelium, our results do not support this. Borch (1987), when analyzing gastric juice, also found a considerable overlap in CEA content between patients with benign and malignant disease. Other tumour-associated antigens have not been found to be specific for gastric and colorectal carcinomas (Skinner and Whitehead, 1982; Fiocca et al., 1988; Ouayang et al., 1987).

Acknowledgements. We thank Inga Finseth, Ellen Hellesylt, Liv Inger Håseth, Mette Myre and Elisabeth Emilsen Mølsted for skillful technical assistance.

References

Borch K., Renvall H., Lundin C. and Wahren B. (1987). Evaluation of gastric carcinoembryonic antigen analysis as an aid during screening for gastric neoplasia in atrophic gastritis. Gut 28, 26-32.

Børmer O.P. (1990). Interference of complement with the binding of carcinoembryonic antigen to solid-phase monoclonal antibodies. J. Immunol. Methods (in press).

Fiocca R., Villani L., Tenti P., Cornaggia M., Ginzi G., Capella C., Prat M., Bussolati G. and Solcia E. (1988). Widespread expression of intestinal markers in gastric carcinoma: a light and electron microscopy study using BD-5 monoclonal antibody. J. Clin. Pathol. 41, 178-187.

Haggarty A., Legler C., Krantz M.J. and Fuks A. (1986). Epitopes of carcinoembryonic antigen defined by monoclonal antibodies prepared from mice immunized with purified carcinoembryonic antigen of HCT-8R cells. Cancer Res. 46, 300-309.

Hammarström S., Shively J., Paxton R.J., Beatty B.G., Larsson A., Gosh R., Børmer O., Buchegger F., Mach J.-P., Burtin P., Fuks A., Kalthoff H., von Kleist S., Grunert F., Matsuoka Y., Kuroki M., Wagener C., Weber T., Yachi A. and Imai K. (1990). Antigenic sites in carcinoembryonic antigen. Summary of an international workshop on the epitope reactivity of monoclonal antibodies against CEA. Cancer Res. (in press).

Hockey M.S., Stokes H.J., Thompson H., Woodhouse C.S., MacDonald F., Fielding J.W.L. and Ford C.H.J. (1984). Carcinoembryonic antigen (CEA) expression and heterogeneity in primary and autologous metastatic gastric tumours demonstrated by monoclonal antibody. Br. J. Cancer 49, 129-133.

Hsu S.M., Raine L. and Fanger H. (1981). Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem. 29, 577-580.

Laurén P. (1965). The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. Acta Pathol. Microbiol. Scand. 64, 31-49.

Lindgren J., Sipponen P., Seppälä K., Tarpilla S., Nordling S., Wahlström T. and Seppälä M. (1979). Carcinoembryonic antigen in endoscopy brush specimens from benign and malignant gastric lesions. Br. J. Cancer 40, 848-855.

Morson B.C., Sobin L.H., Grudmann E., Johansen A., Nagayo I. and Serck-Hanssen A. (1980). Precancerous conditions and epithelial dysplasia in the stomach. J. Clin. Pathol. 33, 711-721.

Nielsen K. and Teglbjærg P.T. (1982). Carcinoembryonic antigen (CEA) in gastric adenocarcinomas. Morphologic patterns and their relationship to a histogenetic classification. Acta. Pathol.

Microbiol. Immunol. Scand. Sect. A 90, 393-396.

Ohuchi N., Wunderlich D., Fujita J., Colcher D., Muraro R., Nose M. and Schlom J. (1987). Differential expression of carcinoembryonic antigen in early gastric adenocarcinomas versus benign gastric lesions defined by monoclonal antibodies reactive with restricted antigen epitopes. Cancer Res. 47, 3565-3571.

Ouyang Q., Vilien M., Juhl B.R., Larsen L.G. and Binder V. (1987). CEA and carbohydrate antigens in normal and neoplastic mucosa. An immunohistochemical study. Acta Pathol. Microbiol. Immunol. Scand. Sect. A 95, 177-183.

Skinner J.M. and Whitehead R. (1982). Tumor markers in carcinoma and premalignant states of the stomach in humans. Eur. J. Cancer Oncol. 18, 227-235.

Stramignoni D. and Coda R. (1985). Distribution of two tumor-associated antigens in gastric lesions as detected by two monoclonal antibodies on tissue sections. Cancer Detec. Prev. 8, 193-206.

Winawer S.J. (1979). Gastric immunoreactivity CEA as a potential marker of malignancy. Gastroenterology 76, 870-879.

Wurster K. and Rapp W. (1979). Histological and immunohistological studies on gastric mucosa. I. The presence of CEA in dysplastic surface epithelium. Pathol. Res. Pract. 164, 270-281.

Accepted November 12, 1989