

# Distribution of Oncofetal Antigen Tumor-associated Glycoprotein-72 Defined by Monoclonal Antibody B72.3

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## ABSTRACT

Murine monoclonal antibody B72.3, prepared against a membrane-enriched extract of human metastatic carcinoma, was reacted with a spectrum of adult and fetal human tissues using avidin-biotin-complex immunohistochemical techniques to evaluate the expression of the reactive tumor associated glycoprotein (TAG)-72 antigen. TAG-72 was shown to be expressed in several epithelial-derived cancers including 94% of colonic adenocarcinomas, 84% of invasive ductal carcinomas of the breast, 96% of non-small cell lung carcinomas, 100% of common epithelial ovarian carcinomas, as well as the majority of pancreatic, gastric, and esophageal cancers evaluated. TAG-72 expression was not observed, however, in tumors of neural, hematopoietic, or sarcomatous derivation, suggesting that the TAG-72 antigen is "pancarcinoma" in nature. Appreciable monoclonal antibody B72.3 reactivity was generally not observed in adult normal tissues, with limited reactivity noted in a few benign lesions of the breast and colon. TAG-72 antigen expression was detected, however, in fetal colon, stomach, and esophagus, thus defining TAG-72 as an oncofetal antigen. TAG-72 has previously been shown to be distinct from carcinoembryonic antigen and other tumor associated antigens. The pancarcinoma distribution and lack of significant reactivity with normal adult tissues of monoclonal antibody B72.3 suggest its potential diagnostic and therapeutic utility for human carcinomas.

## INTRODUCTION

Malignant neoplasms of epithelial cell origin (carcinomas) cause approximately 400,000 deaths/year in the United States and account for greater than 80% of human cancers. Of these cancers, the most common primary sites of carcinoma development include the lung, colon, and breast. Therapeutic intervention is often not successful once metastases of these cancers have occurred; hence, new diagnostic and therapeutic modalities including MAbs<sup>2</sup> to tumor associated antigens are being sought.

Various monoclonal antibodies which recognize tumor associated antigens have been reported (1-5), including MAb B72.3, which is reactive with a high molecular weight ( $M_r \geq 10^6$ ) mucin-like glycoprotein termed TAG-72 (6, 7). This MAb was generated using membrane-enriched extracts of breast carcinoma metastases as an immunogen (8), and previous immunohistochemical studies have demonstrated its reactivity with formalin-fixed, paraffin-embedded human tissues. Approximately 50% of breast carcinomas (9) and 85% of colon carcinomas (10) have previously demonstrated reactivity with MAb B72.3, whereas no reactivity was observed with a limited number of normal adult tissues tested (9, 10). MAb B72.3 has also recently been utilized as an immunocytochemical adjunct for the detection and diagnosis of carcinoma cells in cytology preparations of human effusions (11-13) as well as fine needle aspiration biopsies of tumor masses (14); in addition, TAG-72 antigenic expression has been indicated as a feature differen-

tiating adenocarcinoma from malignant mesothelioma (15). Radioimmunoassays recently developed have shown that MAb B72.3 may be a useful marker for the detection of the TAG-72 antigen in carcinoma patients (16)<sup>3</sup> distinct from the CEA, 19.9, and OC125 antigen detection assays.

Recent investigations have shown that TAG-72 cell surface antigen expression is influenced by the spatial configuration of some carcinoma cell populations (17, 18), and can also be enhanced by incubation of tumor cells with recombinant  $\alpha$ -interferon (19); in addition, <sup>131</sup>I-labeled MAb B72.3 has demonstrated prolonged binding to human colon carcinoma xenografts allowing *in situ* radioimmunodetection in the nude mouse model (20-23). Phase I clinical trials are currently underway to determine the applicability of radiolabeled MAb B72.3 in radioimmunoscintigraphy for the *in situ* detection of human colonic adenocarcinoma.

Johnson *et al.* (6) have recently characterized the high molecular weight ( $M_r \geq 10^6$ ) tumor-associated glycoprotein (TAG-72) recognized by MAb B72.3. The antigen was partially purified from a xenograft of human carcinoma cell line LS174-T using Sepharose CL-4B chromatography and two sequential passages through B72.3 antibody affinity columns. The density of affinity-purified TAG-72 as determined by cesium chloride gradient ultracentrifugation was found to be 1.45 g/ml. Using Western blot techniques and purified TAG-72, the antigen and subsequent antibody interaction was evaluated following treatment with a variety of proteolytic and glycolytic enzymes. Briefly, chondroitinase digestion had no effect, whereas trypsin and neuraminidase changed but did not completely destroy the antigen and or antibody interaction. Chymotrypsin, papain, and pronase affected the antigen such that it was no longer bound by MAb B72.3. These properties as well as detectable presence of blood group-related oligosaccharides and sensitivity to shearing into lower molecular weight forms suggest that TAG-72 is a mucin-like molecule.

We report here that utilizing MAb B72.3 and ABC immunohistochemical techniques prolongation of the primary MAb incubation time increases the sensitivity of detection of the TAG-72 antigen expression in some tissues. The present study was conducted to better define the distribution of the TAG-72 antigen and to determine the potential utility of MAb B72.3 in various aspects of carcinoma patient management. We have thus investigated (a) optimal conditions for detection of TAG-72 antigen expression using immunoperoxidase techniques, (b) TAG-72 antigen expression in a spectrum of neoplasms as well as their respective benign counterparts, and (c) distribution of the TAG-72 antigen in a spectrum of normal adult and fetal tissues.

## MATERIALS AND METHODS

**Monoclonal Antibodies.** The generation, characterization, and purification of murine IgG1 MAb B72.3 has been previously described in detail (8, 20, 23, 24). An isotype identical murine IgG1 MAb (25), MOPC-21 (Litton Bionetics, Charleston, SC) was used under identical

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<sup>2</sup> The abbreviations used are: MAb, monoclonal antibody; TAG, tumor-associated glycoprotein; ABC, avidin-biotin-peroxidase complex; PBS, phosphate-buffered saline; CEA, carcinoembryonic antigen.

<sup>3</sup> T. Klug *et al.*, submitted for publication.

conditions and concentrations on serial sections from each tissue block in each assay as a negative control. Control tissues with known reactivity with MAb B72.3 were also included in each assay and demonstrated staining reactivities which were indistinguishable in all assays.

**Surgical Tissue Specimens.** Paraffin blocks containing surgically resected human tissues were obtained from the Departments of Pathology at the George Washington University Medical Center, Washington, DC, Vanderbilt University Hospital, Nashville, TN, and Duke University Medical Center, Durham, NC. All tissues were fixed in 10% buffered formalin and embedded in paraffin in a routine manner. All tumors were primary lesions from the organs designated (Table 1). Five- $\mu$ m sections from each block were cut and mounted on gelatin-coated glass slides. Fetal tissues were obtained from twins ( $\leq 300$  g each) who died immediately following premature labor. Frozen sections were made from surgically resected tissues that had been immediately frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$ . Frozen sections were cut 5  $\mu$ m in thickness, stored at  $-70^{\circ}\text{C}$ , and fixed in cold acetone (3 min) prior to use. All benign tissues were obtained from patients without a history of cancer.

**Immunohistochemical Methods.** Formalin-fixed, paraffin-embedded human tissues were assayed for TAG-72 antigen expression using a modification of the ABC immunohistochemical methods of Hsu *et al.* (26). Previously utilized methods have been described in detail for MAb B72.3 and immunohistochemical analyses of human tissues (9, 10); however, for this study the primary incubation conditions used (based on titration results) (see "Results") were MAb B72.3 (40  $\mu\text{g}/\text{ml}$ ) overnight at  $4^{\circ}\text{C}$  for formalin-fixed tissues, and MAb B72.3 (40  $\mu\text{g}/\text{ml}$ , 30 min at room temperature) for frozen sections.

Briefly, formalin-fixed paraffin-embedded tissue sections were deparaffinized in xylene and rehydrated in graded ethanols. Frozen sections (cold acetone fixed) and rehydrated formalin-fixed tissues were treated with methanol containing 0.3%  $\text{H}_2\text{O}_2$  for 10 min at room temperature (to block endogenous peroxidase). After rinsing in 1% PBS (pH 7.4), the sections were incubated in 10% normal horse serum (15 min). This latter and all subsequent reagents were diluted in PBS containing 0.1% bovine serum albumin, added at 200  $\mu\text{l}/\text{slide}$  and incubated with the tissue in a sealed moisture chamber. The pretreatment serum was removed and primary MAbs B72.3 and MOPC-21 (negative controls performed on serial sections of each tissue in the same assay) were added at 40  $\mu\text{g}/\text{ml}$  unless otherwise noted. The primary MAbs were removed and the sections were washed in PBS and then incubated with biotinylated horse anti-mouse IgG (H plus L) immunoglobulin (Vector Laboratories, Inc., Burlingame, CA) at a 1:500 dilution for 30 min. Following a PBS rinse, avidin dehydrogenase: biotinylated horseradish peroxidase H complex was added (30 min at room temperature). The slides were rinsed in PBS, and the peroxidase reaction was initiated using 0.06% diaminobenzidine (Sigma) and 0.01% hydrogen peroxide for 5 min. After a final PBS rinse, the sections were counterstained with hematoxylin, dehydrated in ethanol, cleared

in xylene, and mounted under a coverslip using Permount.

**Scoring Method.** Each section was evaluated for the presence of cell-associated (cytoplasmic, apical, or membranous) as well as extracellular diaminobenzidine precipitate (reddish-brown) indicative of primary MAb binding. All tissues were scored using + for clearly positive (brown) and ++ for a dark brown precipitate. Faint blushes ( $\pm$ ) were considered negative, and any reactivity not dark enough to be identified using  $\times 40$  was considered a blush. This scoring procedure should be considered "semiquantitative" at best. The approximate percentage of positive cells for each malignant tissue was scored according to the number of malignant cells positive divided by the total number of malignant cells  $\times 100$ . The approximate percentage of positive cells for benign tumors was scored according to the number of benign epithelial cells positive divided by the total number of benign epithelial cells  $\times 100$ . For normal tissues, the percentage of reactivity is noted for each cell type and is an approximation of the number of cells positive divided by the total number of cells (for that type)  $\times 100$ . Stromal cells, hematopoietic elements including eosinophils, and acellular components (collagen, etc.) were negative in all cases. Mast cell reactivity was disregarded in both MAbs B72.3- and MOPC-21-stained slides because this reactivity was secondary to nonspecific binding of the avidin-biotin reagents as previously reported by Bussolati and others (27).

## RESULTS

**Assay Conditions.** MAb B72.3 was reacted with serial sections of formalin-fixed paraffin-embedded malignant tissues using ABC immunoperoxidase methodology and a variety of MAb B72.3 concentrations, incubation times, and temperatures. An overnight MAb B72.3 incubation (12–14 h) at  $4^{\circ}\text{C}$  was shown to increase the percentage of cellular reactivity as well as assay sensitivity at various B72.3 concentrations; for example, a serous cystadenocarcinoma of the ovary demonstrated 60% carcinoma cell reactivity using a 30-min room temperature incubation, whereas prolongation of the MAb B72.3 incubation to overnight at  $4^{\circ}\text{C}$  increased this reactivity to 90% (Fig. 1). Using the short incubation time the TAG-72 antigen was undetectable at B72.3 (0.03  $\mu\text{g}/\text{ml}$ ), whereas some malignant cells demonstrated reactivity at the same MAb concentration using the overnight incubation. Malignant colon as well as breast tumors demonstrated similar increases in the percentage of tumor cell reactivity using a 12- to 14-h MAb B72.3 incubation. It should be emphasized that the prolonged incubation of MAb at  $4^{\circ}\text{C}$  did not result in any reactivity with normal adjacent epithelial cells, stroma, vessels, etc. Concentrations greater than MAb (40  $\mu\text{g}/\text{ml}$ ) were not utilized because the isotype identical control MAb MOPC-21 reacted nonspecifically (background

Table 1 MAb B72.3 reactivity with human neoplasia

Organ	Histological tumor type	No. tested/ no. reactive	>20% reactive malignant cells
Ovary	Serous cystadenocarcinoma	30/30	14/30 (40) <sup>a</sup>
	Mucinous cystadenocarcinoma	10/10	6/10 (60)
Lung	Squamous cell carcinoma	3/3	1/3
	Adenocarcinoma <sup>b</sup>	28/29	20/29 (69)
	Large cell carcinoma	1/1	0/1
Colon	Adenocarcinoma	51/54 (94)	23/54 (43)
Breast	Invasive ductal carcinoma	37/44 (84)	12/44 (27)
Esophagus	Squamous cell carcinoma	2/2	0/2
Stomach	Adenocarcinoma	3/4	1/4
Pancreas	Adenocarcinoma	3/3	0/3
Prostate	Adenocarcinoma	1/1	0/1
Bladder	Transitional cell carcinoma	0/1	0/1
Skin	Melanoma	0/2	0/2
Lymph nodes	Lymphoma	0/4	0/4
Bone marrow	Leukemia (chronic lymphocytic)	0/1	0/1
Thymus	Thymoma	0/1	0/1
Bone	Osteogenic sarcoma	0/1	0/1
Brain	Glioblastoma multiforme	0/1	0/1

<sup>a</sup> Numbers in parentheses, percentage.

<sup>b</sup> Tissue represents all primary tumors.

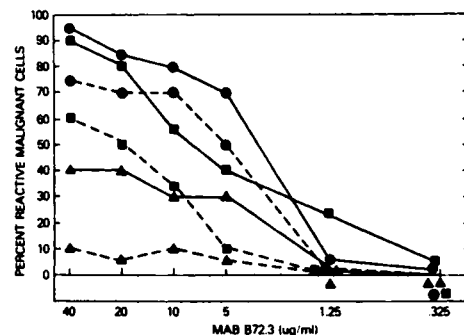


Fig. 1. MAb B72.3 reactivity with carcinomas, overnight versus 30-min primary antibody incubation with formalin-fixed tissues. Serial sections of invasive ductal carcinoma of the breast (●), adenocarcinoma of the colon (▲), and serous cystadenocarcinoma of the ovary (■) were assayed for TAG-72 expression using the ABC immunoperoxidase technique and purified IgG of B72.3 using either a 30-min primary MAb incubation (---) or overnight assay (—). The percentage of positive cells reactive is a semiquantitative value determined by the total number of malignant epithelial cells positive (+ and ++), divided by the total number of malignant epithelial cells  $\times 100$ . For all tissues and MAb concentrations an IgG1 isotype identical primary antibody (MOPC-21) (24) was negative.

staining on epithelium and stroma) at concentrations of 50  $\mu\text{g}/\text{ml}$  or greater. At 40  $\mu\text{g}/\text{ml}$  all tissues were nonreactive with MOPC-21.

**TAG-72 Expression in Human Cancers.** Using the optimal MAb B72.3 incubation conditions described above, a variety of formalin-fixed paraffin-embedded human tumors were analyzed for expression of TAG-72 (Table 1). Adenocarcinomas demonstrated the most TAG-72 expression (Fig. 2, A and C), with 100% (40 of 40) of ovarian, 96% (26 of 27) of lung, 94% (51 of 54) of colon, 84% (37 of 44) of breast, 3 of 3 pancreatic, and 3 of 4 stomach tumors reactive (Fig. 3). Of these, ovarian, colonic, lung, and breast adenocarcinomas demonstrated the highest average percentage of cellular reactivities. Squamous cell carcinomas demonstrated variable reactivity with MAb B72.3, and poorly differentiated squamous cell carcinomas often showed more TAG-72 antigen expression than well-differentiated keratin-producing tumors from the same primary organs (lung, esophagus). At least 5% of the malignant epithelial cells were positive in the majority of adenocarcinomas of the breast (Fig. 3, A and B), colon (Fig. 3C), lung (Fig. 3D), pancreas (Fig. 3E), stomach (Fig. 3F), and ovary as well as esophageal squamous carcinomas.

Human neoplasias of nonepithelial origin failed to demonstrate reactivity with MAb B72.3 (Table 1); these included melanomas, leukemia, lymphomas, a thymoma, sarcoma, and glioblastoma multiforme (high grade astrocytoma).

MAb B72.3 was tested with 54 malignant and 27 benign formalin-fixed paraffin-embedded colonic tissues using ABC immunohistochemical techniques (Fig. 2, A and B). Using the overnight 4°C primary antibody incubation, 94% of primary adenocarcinomas demonstrated reactivity with MAb B72.3; of these, 41% showed  $\geq 25\%$  of the malignant cells expressing the TAG-72 antigen (Fig. 2A). No relationship was observed between Duke's levels, lymph node status, or histological grade and MAb B72.3 reactivity (data not shown). Histological variants including signet ring and mucinous carcinoma demonstrated equivalent reactivities with MAb B72.3. Mucin-secreting tumors, however, often showed TAG-72 expression within secreted mucinous material and glandular lumina.

Heterogeneity of TAG-72 expression was observed between cells of primary tumors as well as between primary, regional, and distant colon carcinoma tumor metastases. Tumor metastases to a variety of tissues including lymph nodes, omentum, liver, soft tissues, bladder, lung, and colon also demonstrated a heterogeneity of TAG-72 expression. No correlation was ob-

served between site of tumor metastasis and percentage of cellular reactivity. MAb B72.3 demonstrated minimal reactivity with benign colon tissues (Fig. 2B), although a single specimen from a patient with active Crohn's disease demonstrated 20% of the epithelial cells reactive with MAb B72.3. All four colon specimens examined without histological abnormalities were negative for TAG-72 expression.

MAb B72.3 was then tested for reactivity with formalin-fixed paraffin-embedded primary invasive ductal carcinomas ( $n = 44$ ) as well as a variety of benign breast lesions ( $n = 20$ ). Eighty-six% of primary breast carcinomas demonstrated expression of the TAG-72 antigen, although only 27% contained  $\geq 25\%$  of the cells reactive with MAb B72.3 (Fig. 2C). No correlation was observed between histological tumor grade, lymph node status, the presence of estrogen receptor, and the expression of the TAG-72 antigen (data not shown). Heterogeneity was also observed in both primary and metastatic lesions in a manner similar to that noted for colon carcinoma.

Despite a prolonged MAb B72.3 incubation time and relatively high concentrations used, a variety of benign breast lesions failed to demonstrate enhanced levels of TAG-72 expression. Six of 20 benign lesions examined (three specimens containing fibrocystic change with hyperplasia and three fibroadenomas) displayed only weak reactivity with MAb B72.3 (Fig. 2D); all six demonstrated  $< 10\%$  of the benign epithelial cells reactive of these lesions. Of particular interest was the finding that cystic disease specimens without hyperplasia failed to react with MAb B72.3. Reactivity with epithelial cells demonstrating apocrine metaplasia was occasionally noted; however, this lesion has a characteristic histological appearance and can be easily differentiated from carcinomas.

**MAb B72.3 Reactivity with Normal Adult Tissues.** As summarized in Table 2, TAG-72 expression in normal tissues was noted in very few cell types from 33 organ and tissue types evaluated. MAb B72.3 reactive cells were noted in only secretory phase endometrial glands (25%), a major salivary gland duct (Fig. 4A, 15%), the esophageal squamous epithelium (1%), the gastric epithelium (1%), respiratory epithelium from the bronchus of the lung (Fig. 4C, 5%), transitional epithelium lining renal pelvis (1%), biliary duct epithelium from liver (1%), and the endocervical glandular epithelium (1%). Of particular interest were major organs including bone marrow (Fig. 4D), lymph nodes (Fig. 4E), liver, spleen, heart and pulmonary and kidney parenchyma which were negative for immunoreactivity with MAb B72.3.

**MAb B72.3 Reactivity with Frozen Human Tissues.** To evaluate the possibility of TAG-72 antigen denaturation by formalin fixation and tissue processing, 5- $\mu\text{m}$  frozen sections were fixed in cold acetone (3 min) and then incubated with MAb B72.3 (40  $\mu\text{g}/\text{ml}$ ). MAb B72.3 demonstrated equivalent reactivity with frozen and fixed human malignant tissues (Table 3). Invasive ductal carcinomas of the breast and adenocarcinomas of the ovary and colon were strongly immunoreactive. TAG-72 expression was not present in benign colon ( $n = 3$ ), breast ( $n = 3$ ), ovary ( $n = 2$ ), lung ( $n = 2$ ), spleen ( $n = 1$ ), and liver ( $n = 1$ ). Minimal reactivity with MAb B72.3 ( $\leq 5\%$ ) was noted in the gastric epithelium. The control MAb MOPC-21 demonstrated no reactivity with serial sections of these frozen tissues assayed simultaneously.

**TAG-72 Expression in Fetal Tissues.** MAb B72.3 demonstrated minimal reactivity with 3 ml of 21 formalin-fixed paraffin-embedded fetal tissues. Reactivity was observed only in tissues of the gastrointestinal tract, including the colon (10% of epithelial cells; Fig. 4G), esophagus (1% of epithelial cells; Fig.

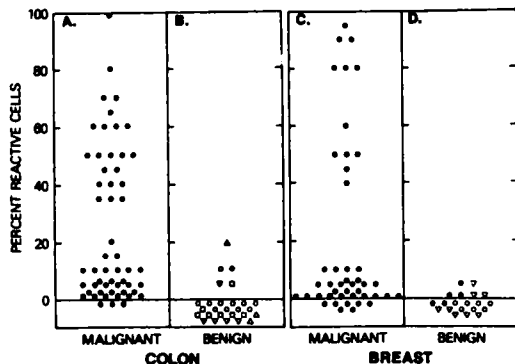


Fig. 2. Reactivity of MAb B72.3 with various colonic (A and B) and breast (C and D) tissues. Purified MAb B72.3 (at 40  $\mu\text{g}/\text{ml}$ ) was reacted with formalin-fixed 5- $\mu\text{m}$  sections of colonic adenocarcinomas (A), tubular adenomas (B,  $\circ$ ), tubulovillous adenomas (B,  $\square$ ), hamartomatous polyp (B,  $\ominus$ ), nonspecific and/or chronic inflammation (B,  $\Delta$ ), Crohn's disease (B,  $\blacktriangle$ ), ulcerative colitis (B,  $\nabla$ ), and normal colon (B,  $\nabla$ ). B72.3 (at 40  $\mu\text{g}/\text{ml}$ ) was also reacted with formalin-fixed 5- $\mu\text{m}$  tissue sections of invasive ductal carcinoma (C,  $\bullet$ ), fibrocystic change (D,  $\circ$ ), fibrocystic change with hyperplasia (D,  $\ominus$ ), and fibroadenomas (D,  $\nabla$ ).

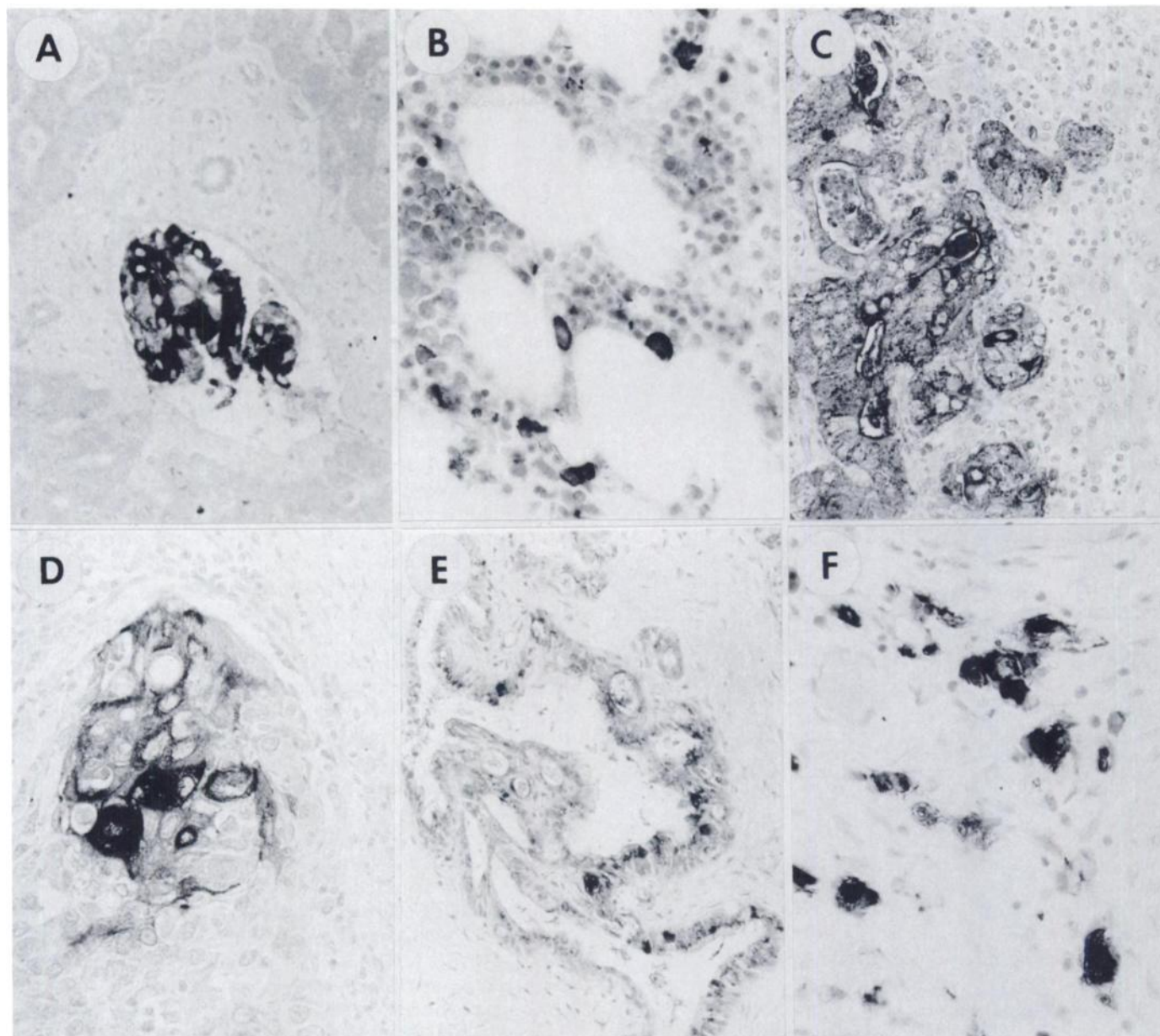


Fig. 3. Immunoperoxidase staining of formalin-fixed paraffin-embedded malignant tumors using MAb B72.3. *A*, invasive ductal carcinoma of the breast, metastatic to vessel within hepatic triad, strongly reactive. Surrounding hepatic cells, biliary duct, and stroma are nonreactive.  $\times 540$ . *B*, metastatic invasive ductal breast carcinoma in bone marrow demonstrates cytoplasmic and membranous reactivity.  $\times 540$ . *C*, adenocarcinoma of the colon, metastatic to the liver demonstrates gland formation and strong MAb reactivity.  $\times 330$ . *D*, squamous cell carcinoma of the lung (primary) with heterogeneous staining reactivity.  $\times 330$ . *E*, pancreatic adenocarcinoma with focal cytoplasmic reactivity.  $\times 220$ . *F*, gastric adenocarcinoma (signet ring histological type) strongly reactive.  $\times 330$ .

4H), and stomach (1% of epithelial cells; Fig. 4I). No reactivity was noted with tissues from other organ systems including the lymphoreticular, cardiovascular, hepatic, pulmonary, neural, muscular, skin, endocrine, and genitourinary tissues.

## DISCUSSION

The advent of hybridoma technology has facilitated the identification of novel antigens, some of which demonstrate selective reactivity with malignant *versus* benign tissues. This has allowed immunological and immunohistochemical phenotyping of malignant cell populations resulting in the identification of pancarcinoma or oncofetal antigens. These include CEA recognized by various MABs (28–31), a  $M_r$  750,000 antigen (CA 125) identified by MAB OC-125 (2, 3), a monosialoganglioside (GICA) recognized by MAB 19-9 (1, 4, 5, 31), and a large heavily glycosylated mucin-like antigen identified with

MAB DU-PAN 2 (32). Other studies have shown that the reactivity of MAB B72.3 is clearly distinct from that of these other MABs (6, 8, 24 and data not shown).<sup>4</sup> Numerous other MABs have been generated which are reactive with mammary carcinoma using immunogens such as breast carcinoma metastasis [e.g., MAB DF3 (33, 34)], mammary carcinoma cells lines [e.g., MAB F36/22 (35, 36)], and milk fat globule membranes [e.g., MABs HMFG 1 and 2 (37, 38)]. The staining pattern of these MABs with malignant breast tissue is similar to that observed with MAB B72.3; however, their reactivities with a variety of other tissues (particularly normal and benign adult tissues) are distinct; for example, MABs DF3 and F36/22 react with normal breast epithelium whereas MAB B72.3 does not. HMFG-2, DF3, and F36/22 react with lactating breast whereas B72.3 does not; in addition, although many of the above MABs do react with high molecular weight glycoproteins, they have

<sup>4</sup> R. Metzgar, personal communication.

Table 2 MAb reactivity with formalin-fixed normal human tissues

Organ system	Positive cell types (% cells reactive)	Negative cell types
Lymphoreticular		
Bone marrow		Myeloid and erythroid precursors, megakaryocytes, bone
Lymph node		Lymphocytes, histiocytes
Spleen		Lymphocytes, reticuloendothelial cells, arteries, erythrocytes
Thymus		Lymphocytes
Gastrointestinal		
Esophagus	Squamous epithelium (<1)	Stroma, smooth muscle, submucosal glands
Stomach	Mucous epithelium (<1)	Mucin secreting epithelium, chief cells, parietal cells
Small intestine		Epithelium, Brunner's glands, stroma, smooth muscle
Colon		Epithelial cells, stroma, smooth muscle
Other		
Heart		Cardiac muscle
Brain		Neural/glia cells
Peripheral nerve		Nerve fibers
Lung	Ciliated respiratory epithelium (5)	Type I and II pneumocytes, macrophages, stroma
Kidney	Transitional epithelium (1)	Tubular epithelium, glomerular epithelial cells, stroma, endothelial cells
Skeletal muscle		Skeletal muscle
Liver	Bile duct epithelium (1)	Hepatocytes, stroma, Kupfer cells
Gall bladder		Bile duct epithelium
Bladder		Transitional epithelium, stroma, smooth muscle
Salivary gland ducts	Large striated duct epithelium (15)	Small intercalated duct epithelium
Salivary gland		Mucinous acinar cells, serous acinar cells
Smooth muscle		
Uterus		
Proliferative	Uterine glandular epithelium (0)	Stroma, smooth muscle
Secretory	Uterine glandular epithelium (25)	
Cervix		
Exo		Squamous epithelium, stroma
Endo	Columnar epithelium (1) (particularly with squamous metaplasia)	Stroma
Ovary		Follicular cells, stroma
Fallopian tube		Ciliated epithelium, smooth muscle, stroma
Prostate		Prostatic epithelium, stroma
Testes		Spermatogenic cells, sertoli cells, Leydig cells, stroma
Endocrine/exocrine		
Thyroid		Follicular cells, parafollicular cells (C-cells), stroma
Parathyroid		Oxyphil cells, chief cells
Adrenal		Cortical cells
Pancreas		Islet of Langerhans/acinar cells

been shown to have different physical, chemical, and immunological properties than TAG-72.

In this study we have utilized immunohistochemical methods with MAb B72.3 to determine the expression of the TAG-72 antigen in a wide range of malignant as well as benign human tissues in an attempt to clearly define the potential of MAb B72.3 in clinical applications. Previous immunohistochemical studies (utilizing a short primary MAb incubation time) have demonstrated MAb B72.3 reactivity with approximately 80% of colon carcinomas (10) and 45% of breast carcinomas (9). Prolongation of the incubation time and doubling of the MAb

concentration utilized has resulted in a marked increase in assay sensitivity (to 94% of colon carcinomas and 84% of breast carcinomas). We have shown here that the TAG-72 antigen demonstrates a pancarcinoma distribution with 96% of lung carcinomas (non-small cell types), 100% of ovarian carcinomas (common epithelial histological cell types), and most pancreatic, gastric, and esophageal carcinomas reactive with MAb B72.3. The finding of a higher percentage of malignant ovarian, lung, and colon tumors reactive with MAb B72.3 in comparison to breast carcinoma is interesting because MAb B72.3 was generated using metastatic breast carcinoma as the immunogen. TAG-72 expression was not found in nonepithelial tumors (including melanomas, sarcomas, tumors of neural crest derivation, leukemia, and lymphoma); very low levels of expression of TAG-72 have been found in some malignant mesotheliomas (15).

Prolongation of assay conditions did not alter MAb B72.3 binding specificity. Low levels of the TAG-72 antigen have been detected in 5 of 27 benign colon and 6 of 20 benign breast tissues; however, histologically normal colonic epithelium as well as nonhyperplastic epithelium of fibrocystic change specimens from these sites remained nonreactive. A wide variety of other normal tissues also failed to demonstrate significant TAG-72 expression (except for one sample of secretory endometrium). Further evaluation of this finding (*i.e.*, TAG-72 expression and uterine epithelium) is warranted. Salivary glands were routinely negative for MAb B72.3 reactivity, although a section through a major gland duct demonstrated expression of the TAG-72 antigen (see Fig. 4). Other tissues which demonstrated  $\leq 1\%$  of the epithelial cells reactive with MAb B72.3 included esophagus, stomach, lung, and endocervix.

TAG-72 antigen expression was not detected despite prolonged incubation with MAb B72.3 in most major organs and bone marrow. This suggests a selective MAb B72.3 binding and potential usefulness in the detection (via radioimmunosciography or serum assays) or therapy of epithelial cancer; indeed, preliminary trials utilizing  $^{131}\text{I}$ -labeled MAb B72.3 have demonstrated no significant *in vivo* binding to normal tissues and selective MAb B72.3 reactivity with tumor allowing visualization of colon carcinoma metastases.<sup>5</sup>

Many antigens recognized by MAbs directed against lymphocyte markers as well as some tumor-associated antigens fail to react with formalin-fixed tissue sections (secondary to antigenic denaturation). Analyses of frozen tissue sections with MAb B72.3 demonstrated TAG-72 expression in malignant breast and colon tumors and no MAb B72.3 reactivity with normal tissues. This finding strongly suggests that the antigenic determinant recognized by MAb B72.3 is not significantly altered during tissue fixation. MAbs which can be utilized with immunoperoxidase techniques and routinely processed (formalin-fixed paraffin-embedded) tissues offer distinct advantages in many cases.

CEA is the prototype oncofetal antigen which has been identified in benign fetal as well as malignant adult tissues. The TAG-72 antigen is distinct from CEA and has recently been purified and characterized as a mucin-like molecule on the basis of its high molecular weight, resistance to chondroitinase digestion, density determination, the presence of blood group-related oligosaccharides, and sensitivity to shearing into lower molecular weight forms (6). The TAG-72 molecule which is immunoreactive with B72.3 may represent (a) a single gene product, (b) a family of multiple gene products, or (c) a family of

<sup>5</sup> D. Colcher *et al.*, manuscript in preparation.

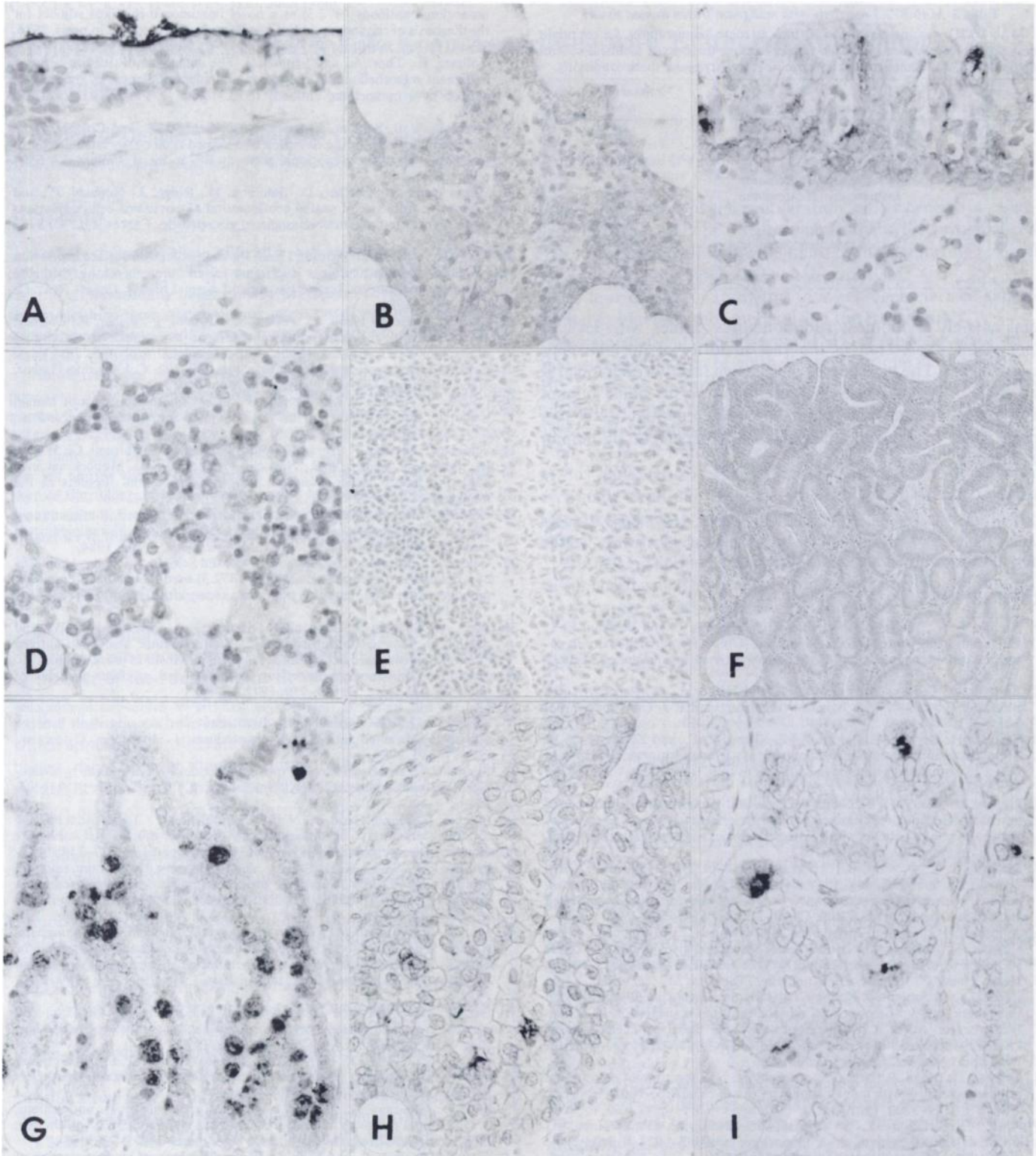


Fig. 4. Immunoperoxidase staining of benign human tissues (adult and fetal) with MAb B72.3. *A*, adult salivary gland duct striated epithelium, apical reactivity.  $\times 540$ . *B*, adult salivary gland, negative for reactivity.  $\times 540$ . *C*, adult bronchial (ciliated respiratory) epithelium.  $\times 540$ . *D*, adult bone marrow, negative for reactivity.  $\times 540$ . *E*, adult lymph node, negative for reactivity.  $\times 330$ . *F*, adenomatous polyp, adult colon, negative for reactivity.  $\times 130$ . *G*, fetal colon, strong focal immunoreactivity.  $\times 330$ . *H*, fetal esophagus, focally reactive.  $\times 540$ . *I*, fetal stomach, focal cytoplasmic reactivity.  $\times 540$ .

molecules representing a single gene product which has been differentially glycosylated. Numerous biochemical and biophysical studies and ultimately cloning of the TAG-72 gene will be required to answer this question. We have demonstrated here that a determinant recognized by MAb B72.3 is expressed in fetal colon, esophagus, and stomach. It is interesting to note that low levels of TAG-72 expression were also noted in the adult counterpart of esophagus and stomach, although it was

not present in normal colonic tissues. Further characterization of the fetal expression of TAG-72 at various gestational ages is in progress<sup>6</sup> and may provide insight into the expression and function of this antigen at various stages of cellular differentiation; hence, the MAb B72.3-defined TAG-72 antigen appears to be a novel oncofetal antigen in that it is expressed in some

<sup>6</sup> J. Lundy *et al.*, manuscript in preparation.

Table 3 MAb B72.3 reactivity with malignant frozen human tissues

MAb B72.3 was incubated for 30 min at room temperature. An overnight assay was not performed on frozen tissues because the isotype identical control MAB (MOPC-21) demonstrated nonspecific reactivity under these conditions.

Organ	Histological diagnosis	% reactive cells
Colon	Adenocarcinoma	10
	Adenocarcinoma	95
Breast	Invasive ductal carcinoma	1
	Invasive ductal carcinoma	30
Ovary	Serous cystadenoma	0
	Serous cystadenocarcinoma	40
	Mucinous cystadenocarcinoma	40

fetal as well as in most carcinomatous tissues with lack of appreciable expression in the vast majority of normal adult counterparts. The high degree of selective reactivity demonstrated here of MAb B72.3 for carcinoma *versus* adult human tissues thus provides the rationale for the potential use of this monoclonal in several areas of the management of human carcinomas.

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