Virchows Arch [Pathol Anat] (1984) 402:389–404

Virchows Archiv A

Immunohistochemical reactivity of a monoclonal antibody prepared against human breast carcinoma

Renato Mariani-Costantini[1], Maria Ines Colnaghi[1], Flavio Leoni[1], Sylvie Mènard[1], Serenella Cerasoli[2], and Franco Rilke[2]

[1] Division of Experimental Oncology A
[2] Anatomical Pathology, Istituto Nazionale per lo Studio e la Cura dei Tumori, Via Venezian 1, I-20133 Milan, Italy

Summary. The reactivity profile of an IgM monoclonal antibody, MBR1, raised against the human breast cancer cell line MCF7, was studied in a variety of human tumours and non-neoplastic tissues by light microscopic immunohistochemistry. The range of reactivity included specific types of non-neoplastic epithelial cells and a number of epithelial tumours. Most mammary carcinomas reacted with MBR1, but adenocarcinomas and squamous carcinomas from different sites were also strongly positive. Different patterns of immunoreactivity were apparent in microscopically normal tissues, in tissues with inflammatory changes and in carcinomas. Heterogeneous staining, despite morphological similarities, was documented in neoplastic and non-neoplastic epithelial cells. The reactivity of MBR1 was different from that reported for other monoclonal antibodies, but revealed similarities to that of monoclonal antibodies and polyclonal sera against human milk fat globule membrane.

Key words: Monoclonal antibody – Immunohistochemistry – Human breast carcinoma – Epithelial cell marker

Findings in recent studies indicate that monoclonal antibodies (MAbs) that identify antigenic determinants associated with human epithelial and/or carcinoma cells have complex and heterogeneous immunohistochemical reactivities. MAbs prepared against human carcinomas react with a variety of tumours and/or with specific types of non-neoplastic cells (Ashall et al. 1982; Finan et al. 1982; Gatter et al. 1982; McGee et al. 1982; Nuti et al. 1982; Kabawat et al. 1983; Stramignoni et al. 1983). With regard to breast carcinoma antigens, studies conducted with polyclonal sera (Heyderman et al. 1979; Imam and Tökés 1981; Sloane and Ormerod 1981) and with murine monoclonal antibodies (Arklie et al. 1981; Taylor-Papadimitriou et al. 1981;

Offprint requests to: F. Rilke at the above address

Foster et al. 1982a and b; Papsidero et al. 1983; Thompson et al. 1983) suggest that a variety of antigenic determinants may be expressed in non-neoplastic breast epithelium, breast carcinomas, and a number of non-neoplastic and neoplastic non-breast epithelial cells.

We have recently reported (Mènard et al. 1983) the generation of three murine MAbs against the human breast carcinoma line MCF7. One of these MAbs, designated MBR1, has been shown to identify a determinant on the sugar moiety of a glycolipidic complex expressed in neoplastic and normal breast epithelial cells (Canevari et al. 1983; Mènard et al. 1983). We employed a sensitive immunoperoxidase technique to define the reactivities of MBR1 in neoplastic and non-neoplastic human tissues, and the results of this immunohistologic study are reported in the present paper.

Material and methods

MAbs. Techniques used for the generation and characterization of murine MAbs reactive with human breast cancer cells are reported elsewhere (Mènard et al. 1983). MBR1 is a murine IgM obtained from fusing splenic lymphocytes, from mice immunized with membrane fractions of the human breast cancer cell line MCF7, with the BALB/c myeloma line P3-X63-AG8-U1. Antibody MBR1 was selected for study because of its specific binding to the membranes of the immunizing cell line in solid phase radioimmunoassay and immunofluorescence. Murine monoclonal IgM B3 (Colnaghi et al. 1982), used as a control in substitution of MBR1, was reactive with murine lymphomas. A pool of MBR1 murine ascites, partially purified by ammonium sulfate precipitation, was used in routine immunoperoxidase screening. The dilution (3 μg protein/ml) was selected at the maximum staining intensity associated with the absence of nonspecific background staining (comparable to that of the control IgM). Reactivities obtained with such a dilution were confirmed using purified MBR1 and corresponded in pattern and intensity to those obtained with purified MBR1 at 1.5 μg/ml.

Tissues. Bouin- or formalin-fixed, paraffin-embedded blocks of non-neoplastic and neoplastic human tissues were selected after pathological review. Five-micron thick sections, collected on albumin-coated slides and heated overnight at 37° C, were used routinely. To exclude loss of antigenic reactivity, the staining of unfixed or acetone-fixed frozen sections was compared to that of routinely processed tissues. Tumours that were MBR1 positive in acetone-fixed or unfixed frozen sections had the same pattern of reactivity, associated with superior visualization of details, in histological sections from routinely paraffin-embedded samples. Methanol fixation of frozen sections resulted in loss of MBR1 immunoreactivity, whereas methanol had no effect on the reactivity of Bouin- or formalin-prefixed tissues even after a 60-min incubation. The permanence of MBR1 immunoreactivity in surgical specimens in the time span between surgery and pathology processing was evaluated in mammary tissues fixed at 1-h intervals from 0 to 5 h after mastectomy. Intense staining was documented after 5 h at room temperature in the surgical pathology laboratory.

Immunoperoxidase technique. In view of its sensitivity (Hsu et al. 1981a), a slight modification of the avidin-biotin-peroxidase technique (Hsu et al. 1981b) was employed. Paraffin-embedded sections were deparaffinized in xylene and rinsed in two changes of absolute ethanol (10 min). Endogenous peroxidase activity was blocked with sequential incubations (15 min each) in two freshly prepared solutions of methanol with 0.3% fresh H_2O_2 at room temperature. After rinsing in phosphate-buffered saline (PBS), pH 7.6, the sections were pretreated with 2% fetal calf serum in Hanks' balanced salt solution for 15 min at room temperature. The same medium was used to dilute all subsequent immunoglobulins (Ig). After removal of the pretreatment serum, the sections were sequentially incubated for 30 min at room temperature with 200 μl/slide of MAb and, after rinsing in two changes of PBS (10 min), with the same amount of biotinylated horse antimurine Ig (Vector, Burlingame, CA, USA) diluted 1/400. After rinsing

Table 1. Immunohistochemical reactivity of MAb MBr1 with breast tissues and breast tumours

Tissue	Cell type	MBr1
Adult female Testing breast	Epithelium	++ (66/80)
	Myoepithelium	− (0/80)
Breast with lactational changes	Epithelium	++ (2/2)
Primary breast carcinoma	Ductal	++ (27/37)
	Lobular	++ (15/18)
Metastatic breast carcinoma	Nodal metastatic cells	++ (12/17)

In brackets: number positive over total tested
++ Diffusely positive − Unreactive

in two changes of PBS, 200 µl/slide of avidin-DH:biotinylated horseradish peroxidase H complex (ABC, Vector) were added for 30 min at room temperature. Following another rinse in two changes of PBS (10 min), the peroxidase reaction was initiated by the addition of 200 µl/slide of 0.06% diaminobenzidine (Sigma, St. Louis, MO, USA) in PBS with 0.01% freshly added H_2O_2 for 5 min at room temperature. The slides were finally rinsed in PBS, lightly counterstained with haematoxylin, dehydrated and mounted under coverslips with resin.

Controls included deletion controls (i.e., with omission of the MAb) and substitution controls, stained with the unrelated murine IgM B3, normalized to the concentration of MBR1. In control frozen sections, staining, probably due to endogenous biotin, was obtained in tissues such as kidney, liver and pancreas treated with biotinylated horse antimurine Ig in the absence of MBR1. Such nonspecific reactivity disappeared in Bouin- or formalin-fixed, paraffin-embedded samples. Dilution curves with MBR1 showed similar reactivity endpoints (between 0.2 and 0.1 µg/ml) for all positive tissues, irrespective of the anatomical site and histology.

Results

Non-neoplastic breast tissues and breast tumours. The reactivity of MBR1 with non-neoplastic epithelia and tumours from the breast is summarized in Table 1. Microscopically normal epithelia of the mammary ductules demonstrated strong staining of the luminal cell membranes and intraluminal secretion product (Fig. 1 a). Interepithelial cell membranes were unreactive. Cytoplasmic staining was also observed, but this was only focal. Myoepithelial cells were negative. In major ducts, positivity was mostly restricted to luminal cell membranes and secretion products, and there was intense staining of apical buds protruding from luminal cells (Fig. 1 b). Mammary epithelia with lactational changes showed foci of strong reactivity distributed to the cytoplasm, apical cell membranes and secretion products. Heterogeneity of immunoreactivity was documented in both resting and lactating breast: positive and negative lobular units were in close proximity to one another in the same tissue section.

The staining pattern of ductal carcinomas varied according to the degree of differentiation. Reactivity of the apical cytoplasm, luminal cell membrane and secretion products was characteristic of well-differentiated carcinomas (Fig. 1 c). In contrast, circumferential labeling of cell membranes and strong cytoplasmic staining, with visualization of intracytoplasmic lumina, were prominent in moderately to poorly differentiated ductal and lobular carcino-

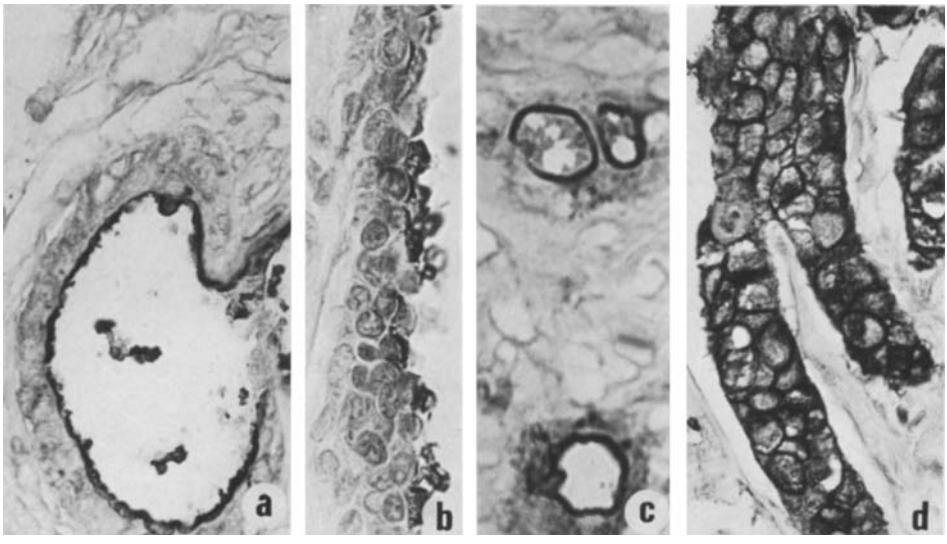

Fig. 1. a Apical staining in a breast ductule (×1650). **b** Reactivity of apical buds in mammary duct epithelium (×1650). **c** Apical staining in a well-differentiated ductal carcinoma (×1650). **d** Membrane and cytoplasmic staining in a poorly differentiated ductal carcinoma (×1050)

mas (Fig. 1d). There was marked variability in the percentages of tumour cells stained in the tumours tested: in some specimens, only isolated cells reacted, whereas in others most cells were strongly labeled. Reactivity with breast cancer metastases in axillary lymph nodes was similar to that of primary carcinomas and demonstrated a pattern of staining with a degree of heterogeneity parallel to that of primary of tumours.

Alimentary system. Nonkeratinized squamous epithelia of the oropharynx and oesophagus (Table 2) revealed a focal, strong, circumferential labeling of cell membranes, associated with weaker cytoplasmic labeling (Fig. 2a). Reactivity was detected in cases with acute inflammation and epithelial hyperplasia and was restricted to the prickle-cell and superficial layers; it was more frequent at the level of epithelial ridges. The basal layer was always negative. Intercellular bridges were well demarcated by diaminobenzidine reaction product (Fig. 2b). The reactivity of squamous cell carcinomas from the oropharynx was similar, except that cytoplasmic staining was more prominent (Fig. 2c).

Serous salivary gland cells and acinar cells of the exocrine pancreas revealed granular cytoplasmic positivity (Fig. 2d), even though intensely reactive acini were often surrounded by negative ones. In salivary glands, the mucous component and myoepithelial cells were unreactive. The epithelium of salivary ducts and, to a lesser extent, of pancreatic ducts demonstrated apical labeling only. Among the benign salivary gland tumours tested, focal apical positivity was detected in the epithelial component of an adenolymphoma. The distribution of the staining was comparable to that observed

Table 2. Immunohistochemical reactivity of MAb MBr1 with tissues and tumours from the alimentary system

Organ	Histology	MBr1	Organ	Histology	MBr1
Tongue	Squamous epithelium	+[a] (5/6)	Small intestine (fetal)	Epithelium	− (0/2)
	Squamous carcinoma	++ (2/4)	Small intestine (adult)	Brunner's glands	+ (3/4)
Salivary glands	Acini and ducts	++ (5/6)	Colon (fetal)	Epithelium	− (0/1)
	Myoepithelium	− (0/6)	Colon (adult)	Epithelium	− (0/12)
	Adenolymphoma	+ (1/1)		Adenocarcinoma (non colloid)	+ (2/3)
	Pleomorphic adenoma	− (0/1)		Adenocarcinoma (colloid)	− (0/3)
	Adenoid-cystic carcinoma	++ (1/1)		Carcinoid	− (0/1)
	Adenocarcinoma	+ (2/3)	Liver	Hepatocytes	−[a] (0/7)
Eso-phagus	Squamous epithelium	+[a] (2/2)		Biliary epithelium	+[a] (1/6)
				Hepatocellular carcinoma	− (0/1)
Stomach	Epithelium	+[a] (5/6)			
	Adenocarcinoma	++ (1/2)	Gall bladder	Epithelium	− (0/2)
	Carcinoid	− (0/1)	Pancreas	Acinar cells	++ (10/10)
	Lymphoma	− (0/1)		Ducts	+ (1/10)
				Adenocarcinoma	− (0/5)

In brackets: Number positive over total tested.
+ Focally positive; ++ diffusely positive; − negative; [a] reactivity associated with inflammatory changes

in normal salivary duct epithelium. A pleomorphic adenoma of the parotid gland was negative. With regard to the malignant salivary tumours tested, apical staining was found in an adenoid-cystic carcinoma (Fig. 2e). The predominant distribution was apical and secretory, but cytoplasmic reactivity was also present. In poorly differentiated salivary adenocarcinomas, there was only focal membrane and cytoplasmic positivity.

In the stomach, reactivity consisted of focal, foamy cytoplasmic staining of goblet cells of intestinal metaplasia and antral gland cells, associated with week apical labeling of surface epithelial cells. A similar, weak labeling was detected in some of Brunner's glands in the duodenum. In contrast, normal colonic epithelium and fetal small and large intestine (30 weeks of gestational age) were negative. Adult bile duct epithelium showed foci of apical reactivity in only one of six cases, in association with acute inflammation. Foci of strong apical, membrane, and cytoplasmic staining were observed in gastric and colonic adenocarcinomas of the non-colloid type

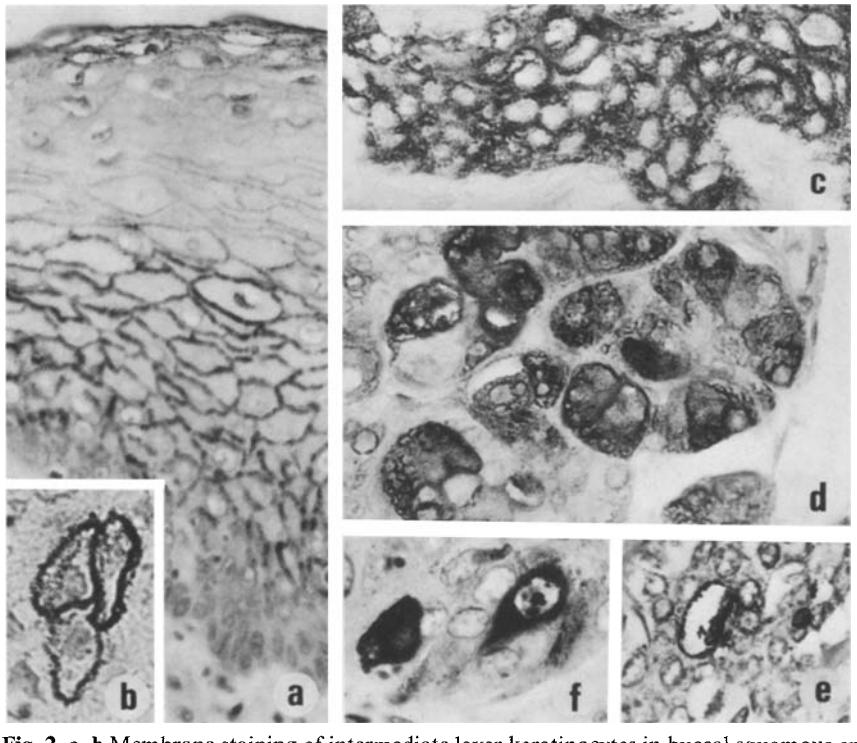

Fig. 2. a, b Membrane staining of intermediate layer keratinocytes in buccal squamous epithelium (**a**, ×1050; **b**, ×1650). **c** Membrane and cytoplasmic staining in a squamous cell carcinoma of the tongue (×1050). **d** Membrane and cytoplasmic staining in serous salivary cells (×1650). **e** Apical labeling in an adenoid cystic salivary carcinoma (×1605). **f** Cytoplasmic reactivity in colonic adenocarcinoma cells (×1650)

(Fig. 2f). The colloid carcinomas tested were negative. Gastrointestinal carcinoids, pancreatic and hepatocellular carcinomas were also unreactive.

Respiratory system. No reactivity was detected in microscopically normal tissues from the respiratory system (Table 3). However, foci of reactivity appeared in association with inflammatory changes. In bronchial epithelium, apical and vesicular epinuclear staining were detected in a specimen with acute inflammation. In alveolar respiratory epithelium, strong periluminal labeling of pneumocytes was consistently demonstrated in association with fibrosis and chronic inflammation either in the adjacencies of primary and metastatic carcinomas or in tissues from patients with chronic obstructive pulmonary disease (Fig. 3a). Positivity was especially prominent in plump type II pneumocytes. Of the respiratory tumours tested, a variety of lung carcinomas was positive. In adenocarcinomas, apical and secretion product staining of neoplastic glands was associated with membrane and cytoplasmic labeling (Fig. 3b). In squamous cell carcinomas, circumferential membrane labeling was associated with foamy, cytoplasmic reactivity as in squamous

Table 3. Immunohistochemical reactivity of MAb MBr1 with tissues and tumours from the respiratory and urinary systems

Organ	Histology	MBr1	Organ	Histology	MBr1
Respiratory System			*Urinary System*		
Maxillary sinus	Colloid carcinoma	− (0/1)	Kidney (fetal)	Glomeruli and tubules	− (0/2)
Larynx	Squamous epithelium	− (0/1)	Kidney (adult)	Glomeruli	− (0/5)
Lung (fetal)	Pulmonary tubules, stroma	− (0/1)		Proximal tubules	− (0/5)
				Distal and collecting tubules	+ (5/5)
Lung (adult)	Bronchial epithelium	+[a] (1/3)		Renal carcinoma	− (0/7)
	Alveolar epithelium	+[a] (13/16)	Bladder	Urothelium	+[a] (1/2)
	Adenocarcinoma	+ + (4/6)		Transitional cell carcinoma	+ (1/2)
	Squamous carcinoma	+ + (3/4)			
	Small cell carcinoma	+ (3/5)			
	Bronchiolo-alveolar carcinoma	+ (1/2)			
	Large cell carcinoma	+ (2/4)			
	Adenocarcinoid	− (0/1)			

In brackets: Number positive over total tested
+ Focally positive; + + diffusely positive; − negative; [a] reactivity associated with inflammatory changes

cell carcinomas from other sites. In small cell and large cell carcinomas, cytoplasmic and membrane labeling was restricted to small clusters of tumour cells in some specimens only.

Urinary system. Foci of strong reactivity were routinely detected in the epithelium of distal and collecting renal tubules (Fig. 3c, Table 3). The distribution was predominantly apical and, to a lesser extent, cytoplasmic. Glomeruli and proximal tubules were negative. Fetal kidney (30 weeks of gestational age) was also unreactive. In transitional cell epithelium, apical labeling of the luminal cells was detected in association with inflammation in areas adjacent to a transitional cell carcinoma. Among the urinary system tumours tested, renal carcinomas were consistently unreactive, whereas strong cytoplasmic and membrane staining was observed in a transitional cell carcinoma of the urinary bladder, although only in a few cells.

Reproductive system. Reactivity was detected in a variety of epithelia of the female and male genital systems (Table 4). Squamous epithelium from

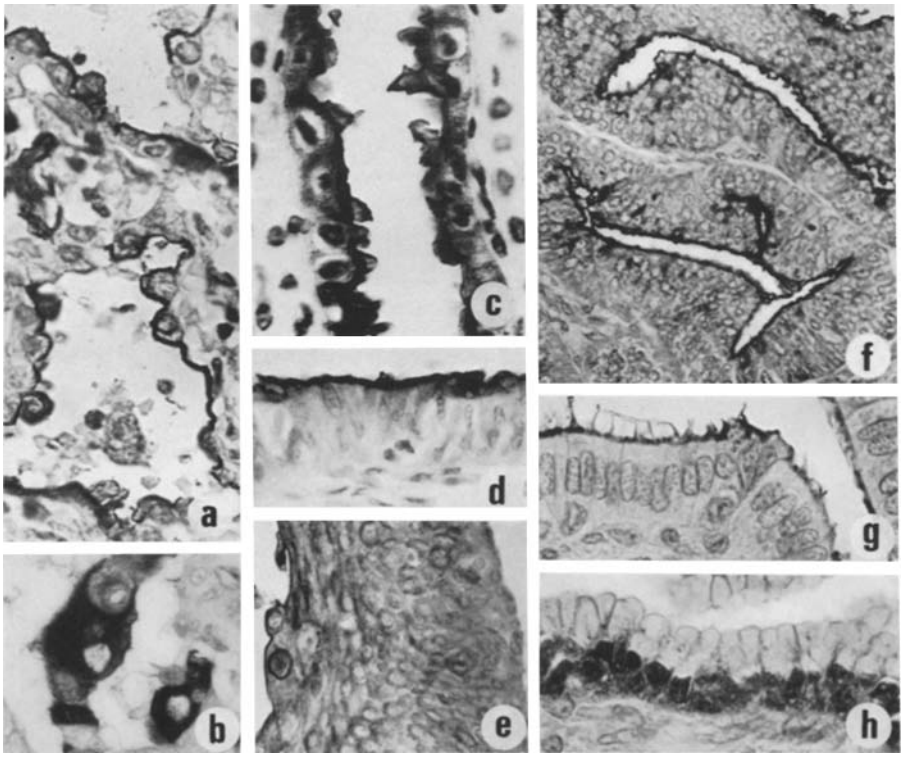

Fig. 3. **a** Apical staining of pneumocytes in thickened alveolar septa (×1650). **b** Cytoplasmic reactivity in pulmonary adenocarcinoma cells (×1650). **c** Apical and cytoplasmic reactivity in a collecting renal tubule (×1650). **d** Apical reactivity in oviduct epithelium (×1650). **e** Membrane and cytoplasmic reactivity in in situ squamous cell carcinoma of the uterine cervix (×660, curettings). **f** Apical reactivity in a well-differentiated endometrial adenocarcinoma (×1050). **g** Apical and **h** epinuclear staining in mucinous ovarian cystadenocarcinomas (×1650)

the vagina and exocervix consistently revealed membrane labeling of cells in intermediate and superficial layers. The basal layer was unreactive. All the specimens examined had chronic and/or acute inflammatory changes. Only apical labeling was seen in endocervical epithelium. In the endometrium, isolated glands, predominantly with a secretory histological pattern, had apical and secretory labeling associated with foamy cytoplasmic reactivity. The epithelium of the oviducts showed apical labeling only (Fig. 3d). Intense reactivity of buds originating from the apical cytoplasm was detected in endometrial and oviduct epithelia. This pattern of distribution was comparable to that in the epithelium of mammary ducts. Ovarian mesothelium, stroma and follicles were negative.

A number of female genital carcinomas was consistently reactive with MBR1. In cervical squamous carcinomas, membrane and cytoplasmic labeling were present in in situ as well as in infiltrating lesions (Fig. 3e). Predominantly apical positivity was characteristic of neoplastic glands in endometrial

Table 4. Immunohistochemical reactivity of MAb MBr1 with tissues and tumours from the reproductive and integumentary systems

Organ	Histology	MBr1	Organ	Histology	MBr1
Reproductive system					
Vagina	Squamous epithelium	++[a] (1/1)	Epididymis	Epithelium	++ (1/1)
Uterus	Exocervical epithelium	++[a] (5/5)	Ductuli efferentes	Epithelium	++ (1/1)
	Squamous carcinoma in situ	++ (5/5)	Seminal vesicles	Epithelium	++ (1/1)
	Invasive squamous carcinoma	++ (1/1)	Testis	Spermatogonia	– (0/2)
	Endocervical epithelium	+ (4/10)		Spermatocytes	– (0/2)
	Endometrial glands	+ 64/14)		Spermatozoa	– (0/2)
	Endometrial carcinoma	++ (2/3)		Seminal epithelium	– (0/2)
	Myometrium	– (0/5)		Leydig cells	– (0/2)
	Leiomyoma	– (0/1)		Seminoma	– (0/1)
Placenta	Chorionic villi	– (0/1)		Embryonal carcinoma	– (0/1)
	Decidua	– (0/1)	*Integumentary system*		
Oviduct	Epithelium	++ (4/5)	Skin	Epidermis (malphigian and superficial layers)	+[a] (10/10)
Ovary	Stroma	– (0/6)		Basal cell carcinoma	– (0/5)
	Ovarian follicles	– (0/6)		Squamous carcinoma	+ (1/3)
	Ovarian mesothelium	– (0/6)		Trichilemmoma	– (0/1)
	Endometrioid carcinoma	++ (3/4)		Trichoepithelioma	– (0/1)
	Serous cystadenocarcinoma	++ (2/5)		Sweat glands (eccrine)	++ (10/10)
	Mucinous cystadenocarcinoma	++ (2/5)		Sweat glands (apocrine)	+ (1/1)
Prostate	Adenomatous glands	+ (1/3)		Sebaceous glands	+ (1/1)

In brackets: Number positive over total tested
+ Focally positive; ++ diffusely positive; – negative; [a] reactivity associated with inflammatory changes

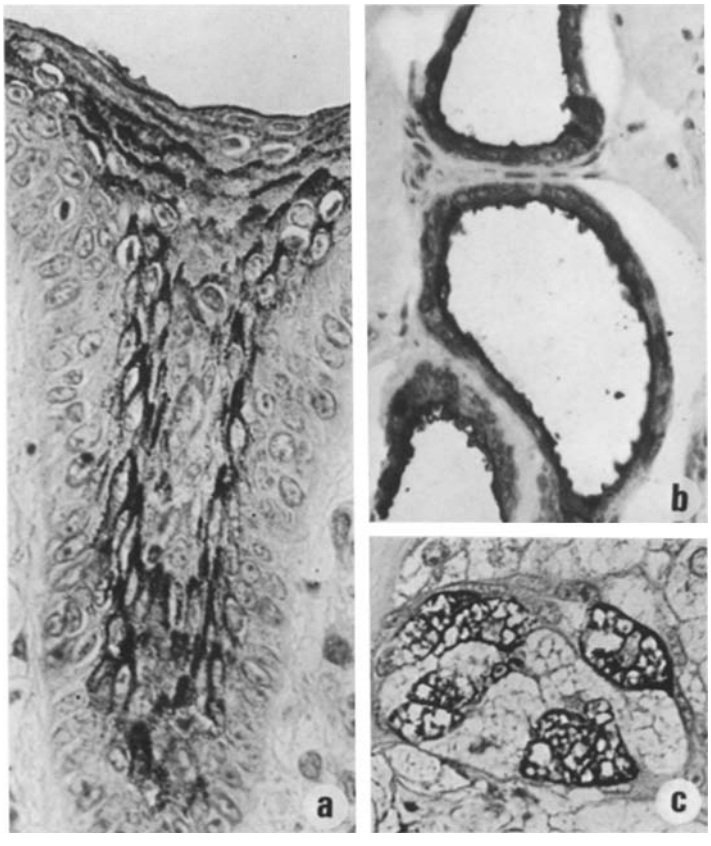

Fig. 4. **a** Staining of malpighian and superficial layers in epidermis (×1650). **b** Apical and cytoplasmic staining in a Moll gland duct (×1030). **c** Staining of sebaceous gland cells (×1650)

adenocarcinomas (Fig. 3f). The common histological varieties of ovarian epithelial tumours were all positive. The staining of ovarian endometrioid carcinomas was similar to that of endometrial adenocarcinomas. In serous cystadenocarcinomas, staining was distributed along cell membranes and was often associated with cytoplasmic reactivity. In some mucinous cystadenocarcinomas, apical cell membranes and extracellular secretion product were stained (Fig. 3g), but in one specimen reactivity was restricted to epinuclear vesicles (Fig. 3h).

With regard to the male genital system, strong immunoreactivity was associated with the epithelium of the ductuli efferentes, epididymis and seminal vesicles. In prostates with adenomatous hyperplasia, only some glands were stained. In all cases, secretion product and apical staining, with strong reactivity of apical buds, was prominent. Seminal epithelium, interstitial cells and spermatozoa were unreactive. One seminoma and one embryonal carcinoma tested were similarly negative.

Table 5. Immunohistochemical reactivity of MAb MBr1 with tissues and tumours from the endocrine, hemolymphatic, nervous and musculoskeletal systems and from the serous cavities

Organ	Histology	MBr1	Organ	Histology	MBr1
Hemolymphatic system			*Central nervous system*		
Spleen	White pulp	– (0/4)	Cerebral cortex	Neurons	– (0/1)
	Red pulp	– (0/4)		Glial cells	– (0/1)
Bone marrow	Erythroid lineage	– (0/22)		Glioblastoma multiforme	– (0/1)
	Myeloid lineage	– (0/22)			
	Megakaryocytic lineage	– (0/22)	*Peripheral nervous system*		
			Peripheral nerves	Schwannoma	– (0/2)
Lymph node	Reactive	– (0/7)		Paraganglioma	– (0/1)
Endocrine system			*Musculoskeletal system*		
Adult adrenal gland	Cortex	– (0/2)	Cartilage	Adult	– (0/5)
	Medulla	– (0/2)		Fetal	– (0/1)
	Neuroblastoma	– (0/1)	Skeletal muscle	Adult	– (0/5)
Fetal adrenal gland	Cortex	– (0/1)		Fetal	– (0/1)
				Rhabdomyosarcoma	– (0/1)
Pancreas	Islets of Langerhans	– (0/10)	Connective tissue	Adult	– [a]
Thyroid	Follicles	– (0/7)		Fetal	– (0/2)
	Parafollicular cells	– (0/7)		Malignant fibrous histiocytoma	– (0/1)
	Adenoma	– (0/1)			
	Follicular carcinoma	– (0/1)	*Serous cavities*		
			Mesthelium	Mesothelial cells	– (0/5)
	Papillary carcinoma	– (0/1)		Mesothelial cysts	– (0/2)
	Medullary carcinoma	– (0/1)			

In brackets: Number positive over total tested
– Negative; [a] negative in all tested tissues

Integumentary system. Membrane and cytoplasmic staining were detected in some epidermal cells, restricted to the malpighian and superficial layers (Table 4). The basal layer was unreactive. Most reactive cells were localized at the level of epidermal ridges and associated with acute and chronic inflammation and epidermal hyperplasia (Fig. 4a). In the epidermal adnexa, a consistent reactivity was found in apocrine and eccrine sweat glands, excluding Moll's glands of the eyelid (Fig. 4b). Membrane and cytoplasmic stain-

ing were equally strong in sweat acini, whereas apical labeling predominated in sweat ducts. In sebaceous glands, strong membrane and foamy cytoplasmic reactivity was detected in isolated cells (Fig. 4c). Among the skin tumours tested, basal cell carcinoma, trichilemmoma and trichoepithelioma were negative, except that weak reactivity was focally detected in luminal structures or in squamoid areas. Membrane and cytoplasmic reactivity was detected in a squamous cell carcinoma of the skin. Malignant melanomas were consistently unreactive.

Other systems. Tissues and tumours from the hemolymphatic, endocrine, musculo-skeletal, central and peripheral nervous systems were negative (Table 5). In addition, it should be pointed out that pleural and peritoneal mesothelium, including activated mesothelium in mesothelial cysts, was also unreactive.

Discussion

The results of an indirect immunofluorescence assay, performed on a restricted sample of tissues, previously demonstrated MBR1 binding in non-neoplastic and neoplastic breast epithelial cells and in apocrine sweat glands (Mènard et al. 1983). Using the highly sensitive ABC immunoperoxidase technique (Hsu et al. 1981a and 1981b), and studying a variety of human tissues and tumours, we found a more complex spectrum of reactions. The immunoperoxidase reactivity of MBR1 included specific types of non-neoplastic cells and a number of epithelial tumours. Differences in staining according to anatomical site and histology were apparent in microscopically normal epithelia, in epithelia with inflammatory changes, and in carcinomas. Comparisons of the reactivity of non-neoplastic epithelia with that of carcinomas in specific organs yielded five possible associations: 1) in the pancreas and kidney, positivity was detected in specific epithelial cell types, but carcinomas were unreactive; 2) in a variety of epithelia, such as those of the breast, endometrium and salivary glands, positivity was detected in normal and cancer cells, and was often enhanced in tumours; 3) in the respiratory, gastric and stratified squamous epithelia significant positivity was apparent in association with inflammatory changes and/or hyperplasia, and was often enhanced in carcinomas; 4) in the large intestine and the ovary, positivity was detected in carcinomas only; 5) in endocrine glands and the liver (hepatocytes and hepatocellular tumours), non-neoplastic and carcinoma cells were unreactive.

A shift from apical labeling in benign epithelial cells to circumferential membrane and cytoplasmic reactivity in neoplastic cells was consistently observed in a variety of sites with non-squamous epithelia, most notably in the breast. A similar alteration in antigenic reactivity was previously documented with polyclonal sera against human milk fat globule antigens (Imam and Tökés 1981; Sloane and Ormerod 1981) and T antigen (Stegner et al. 1981) and with a variety of lectins that bind carbohydrate moieties (Howard and Batsakis 1981; Stegner et al. 1981; Franklin 1983). With re-

gard to squamous cell carcinomas, MBR1 membrane reactivity comparable to that of non-neoplastic squamous cells persisted, but was associated with strong cytoplasmic staining.

Heterogeneity of staining despite apparent morphological identity was present in non-neoplastic epithelia and carcinomas. The specificity of the labeling for well-defined histological structures, its persistence in serial sections, and the presence of a similar heterogeneity in frozen sections ruled out the possibility of a fixation artifact. Heterogeneity of antigenic phenotypes has been extensively documented within cell lines and tissue sections of human mammary cancer (Horan Hand et al. 1983). Cell cycle-related changes in membrane antigenic expression might contribute to such heterogeneity (Kufe et al. 1983).

The distribution of the MBR1 immunohistochemical reactivity in epithelia and carcinomas might reflect functional or evolutionary relationships between molecules in different tissues and tumours, but could also be explained by the fortuitous presence of similar or identical epitopes on different molecules in different cell types (Lane and Koprowski 1982; Nig et al. 1982). Any interpretation must wait for the data on the nature and functional roles of reactive molecules recognized in different tissues. Several authors have reported results of immunohistological studies with MAbs to breast epithelium or breast carcinoma. The immunohistochemical reactivity of MBR1 was distinct from those of other MAbs, but was quite comparable to those of MAbs and polyclonal sera against human milk fat globule membrane (MFGM) preparations. Arklie et al. (1981) and Gatter et al. (1982) published the immunoperoxidase reactivities of two MAbs that recognized MFGM glycoproteins, previously designated 1.10.F3 and 3.14.A3 and presently HMFG1 and HMFG2 (Taylor-Papadimitriou et al. 1981; Burchell and Taylor-Papadimitriou 1983). Both were similar to MBR1 in that they reacted with normal and malignant breast epithelial cells, salivary and pancreatic acinar cells, distal and collecting renal tubles, and endometrial glands. In contrast, both could be distinguished from MBR1 in that they stained normal colonic and thyroid epithelium and renal carcinomas. In addition, HMFG1 clearly differed from MBR1 in that it reacted with smooth muscle cells. The immunoperoxidase reactivities of four other MAbs against MFGM have been described in detail (Foster et al. 1982a and 1982b). Two of these MAbs (LICR LON/M3 and LICR LON/M18) could be distinguished from MBR1 in that they reacted with mesenchymal cells (LICR LON/M3 with smooth and skeletal muscle, LICR LON/M18 with brain capillaries) and adrenal cortical cells. The range of reactivity of the other two MABs (LICR LON/M24 and LICRLON/M8) was restricted to epithelial cells but also differed from that of MBR1. Antibody LICR LON/M24 labeled normal colonic, tracheal and male germinal epithelia and the interepithelial cell membranes in mammary ducts. Antibody LICR LON/M8 had a reactivity profile similar to that of MBR1 in normal breast epithelium, but clearly differed in that it stained hepatocytes, spermatozoa and their precursors, and normal tracheal, thyroid and colonic epithelium.

The immunohistological profile of a polyclonal serum against MFGM,

which identified a major component termed "EMA" (Heyderman et al. 1979; Sloane and Ormerod 1981), had remarkable similarities to the reactivity of MBR1. The range of reactivity of the anti-EMA serum encompassed epithelia and carcinomas reactive with MBR1, but was broader and also included mesothelial cells. Immunoreactivity changes associated with inflammation were documented in specific sites (i.e., squamous epithelia and urothelium). The reactivity of another polyclonal serum that identified an MFGM glycoprotein (Imam and Tökés 1981) was also similar to that of MBR1 in breast epithelium and breast tumours. Two MAbs raised to the MCF7 cell line (Papsidero et al. 1983; Thompson et al. 1983) also reacted with normal and malignant breast epithelial cells, but their reported ranges of extramammary reactivity were different from that of MBR1.

A variety of MAbs to breast carcinoma appeared, to a greater or lesser extent, unrelated to MBR1. Three MAbs to an estrogen-induced protein (Ciocca et al. 1983) reacted by ABC immunoperoxidase with a number of epithelia that we found positive with MBR1. However, they reacted with a minority rather than with the majority of the non-neoplastic and neoplastic breast specimens tested. Some non-epithelial tissues were also positive. Murine MAbs prepared against metastatic mammary carcinomas (Colcher et al. 1981; Nuti et al. 1982) had restricted binding to non-neoplastic mammary epithelium by immunoperoxidase assay on tissue sections. A human MAb (Schlom et al. 1980; Teramoto et al. 1982) was also reported to have little reactivity with non-neoplastic breast epithelium. The Cal MAb (Ashall et al. 1982), which strongly reacted with breast carcinoma cells, did not react with most non-neoplastic epithelia (McGee et al. 1982), but stained a number of non-epithelial tumours.

In conclusion, we showed that the range of reactivity of MBR1 included a variety of epithelial and carcinomas, that there was association between immunoreactivity and inflammatory changes in some non-neoplastic epithelia, and that the major differences between non-neoplastic and neoplastic MBR1-positive epithelial cells were related to variations in the intensity and distribution of the staining. We also showed that there was marked heterogeneity of MBR1 binding in neoplastic and non-neoplastic epithelial cells, despite morphological identity at the light microscopic level. Finally, we demonstrated that the immunoperoxidase reactivity of MBR1 was distinct from that reported for other MAbs against breast epithelium and breast carcinoma, but had similarities to that of MAbs and polyclonal sera to MFGM.

Acknowledgements. This study was supported by contract no. 82.01335.96 (Finalized Project "Control of Tumor Growth") from the Consiglio Nazionale delle Ricerche, Rome.

References

Arklie J, Taylor-Papadimitriou J, Bodmer W, Egan M, Millis R (1981) Differentiation antigens expressed by epithelial cells in the lactating breast are also detectable in breast cancers. Int J Cancer 28:23–29

Ashall F, Bramwell ME, Harris H (1982) A new marker for human cancer cells. 1. The Ca antigen and the Cal antibody. Lancet ii:1–6

Burchell J, Taylor-Papadimitriou J (1983) Complexity of antigenic determinants recognized by monoclonal antibodies HMFG-1 and HMFG-2. In: Proceedings, Breast Cancer Research Conference, Denver, p 52

Canevari S, Fossati G, Balsari A, Sonnino S, Colnaghi MI (1983) Immunochemical analysis of the determinant recognized by a monoclonal antibody (MBR1) which specifically binds to human mammary epithelial cells. Cancer Res 43:1301–1305

Ciocca DR, Adams DJ, Edwards DP, Bjerke RJ, McGuire WL (1983) Distribution of an estrogen-induced protein with a molecular weight of 24,000 in normal and malignant human tissues and cells. Cancer Res 43:1204–1210

Colcher D, Horan Hand P, Nuti M, Schlom J (1981) A spectrum of monoclonal antibodies reactive with human mammary tumor cells. Proc Natl Acad Sci (USA) 78:3199–3203

Colnaghi MI, Ménard S, Tagliabue E, Della Torre G (1982) Heterogeneity of the natural humoral anti-tumor immune response in mice as shown by monoclonal antibodies. J Immunol 128:2757–2762

Final PJ, Grant RM, de Mattos C, Takei F, Berry PJ, Lennox ES, Bleehen NM (1982) Immunohistochemical techniques in the early screening of monoclonal antibodies to human colonic epithelium. Br J Cancer 46:9–17

Foster CS, Dinsdale EA, Edwards PAW, Neville AM (1982a) Monoclonal antibodies to the human mammary gland. II. Distribution of determinants in breast carcinomas. Virchows Arch [Pathol Anat] 394:295–305

Foster CS, Edwards PAW, Dinsdale EA, Neville AM (1982b) Monoclonal antibodies to the human mammary gland. I. Distribution of determinants in non-neoplastic mammary and extra mammary tissues. Virchows Arch [Pathol Anat] 394:279–293

Franklin WA (1983) Tissue binding of lectins in disorders of the breast. Cancer 51:295–300

Gatter KC, Abdulaziz Z, Beverley P, Corvalan JRF, Ford C, Lane EB, Mota M, Nash JRG, Pulford K, Stein H, Taylor-Papadimitriou J, Woodhouse C, Mason DY (1982) Use of monoclonal antibodies for the histopathological diagnosis of human malignancy. J Clin Pathol 35:1253–1267

Heyderman E, Steele K, Ormerod G (1979) A new antigen on the epithelial membrane: its immunoperoxidase localization in normal and neoplastic tissue. J Clin Pathol 32:35–39

Horan Hand P, Nuti M, Colcher D, Schlom J (1983) Definition of antigenic heterogeneity and modulation among human mammary carcinoma cell populations using monoclonal antibodies to tumor-associated antigens. Cancer Res 43:728–735

Howard DR, Batsakis JG (1980) Cytostructural localization of a tumor associated antigen. Science 210:201–203

Hsu SM, Raine L, Fanger H (1981a) A comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. Am J Clin Pathol 75:734–738

Hsu SM, Raine L, Fanger H (1981b) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques. J Histochem Cytochem 29:577–580

Imam A, Tökés Z (1981) Immunoperoxidase localization of a glycoprotein on plasma membrane of secretory epithelium from human breast. J Histochem Cytochem 29:581–584

Kabawat SE, Bast RC, Welch WR, Knapp RC, Colvin RB (1983) Immunopathologic characterization of a monoclonal antibody that recognizes common surface antigens of human ovarian tumors of serous, endometrioid and clear cell types. Am J Clin Pathol 79:98–104

Kufe DW, Nadler L, Sargent L, Shapiro H, Hand P, Austin F, Colcher D, Schlom J (1983) Biological behavior of human breast carcinoma-associated antigens expressed during cellular proliferation. Cancer Res 43:851–857

Lane D, Koprowski H (1982) Molecular recognition and the future of monoclonal antibodies. Nature 296:200–202

McGee JO'D, Woods JC, Ashall F, Bramwell ME, Harris H (1982) A new marker for human cancer cells. 2. Immunohistochemical detection of the Ca antigen in human tissues with the Cal antibody. Lancet ii:7–11

Mènard S, Tagliabue E, Canevari S, Fossati G, Colnaghi MI (1983) Generation of monoclonal antibodies reacting with normal and cancer cells of human breast. Cancer Res 43:1295–1300

Nigg EA, Walter G, Singer SJ (1982) On the nature of crossreactions observed with antibodies directed to defined epitopes. Proc Natl Acad Sci (USA) 79:5939–5943

Nuti M, Teramoto YA, Mariani-Costantini R, Horan Hand P, Colcher D, Schlom J (1982) A monoclonal antibody (B 72.3) defines patterns of distribution of a novel tumor associated antigen in human mammary carcinoma cell populations. Int J Cancer 29:539–545

Papsidero DL, Croghan GA, O'Connell JM, Valenzuela LA, Nemoto T, Chu MT (1983) Monoclonal antibodies (F36/22 and M7/105) to human breast carcinoma. Cancer Res 43:1741–1747

Schlom J, Wunderlich D, Teramoto YA (1980) Generation of human monoclonal antibodies reactive with human mammary carcinoma cells. Proc Natl Acad Sci (USA) 77:6841–6845

Sloane JP, Ormerod MG (1981) Distribution of epithelial membrane antigen in normal and neoplastic tissues and its value in diagnostic tumor pathology. Cancer 47:1786–1795

Stegner HE, Fischer K, Poschmann A (1981) Immunohistochemical localization of Thomsen-Friedenreich antigen in normal and malignant breast tissue using peroxidase-antiperoxidase technique. Tumor Diagnostik 3:127–130

Stramignoni D, Bowen R, Atkinson BF, Schlom J (1983) Differential reactivity of monoclonal antibodies with human colon adenocarcinomas and adenomas. Int J Cancer 31:543–552

Taylor-Papadimitriou J, Peterson JA, Arklie J, Burchell J, Ceriani RL, Bodmer WF (1981) Monoclonal antibodies to epithelium-specific components of the human milk fat globule membrane: production and reaction with cells in culture. Int J Cancer 28:17–21

Teramoto YA, Mariani R, Wunderlich D, Schlom J (1982) The immunohistochemical reactivity of a human monoclonal antibody with tissue sections of human mammary tumors. Cancer 50:241–249

Thompson CH, Jones SL, Whitehead RH, McKenzie IFC (1983) A human breast tissue-associated antigen detected by a monoclonal antibody. JNCI 70:409–419

Accepted November 28, 1983