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The immunohistochemical reactivity of a new anti-epithelial monoclonal antibody (MAb b-12) against breast carcinoma and other normal and neoplastic human tissues

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Summary. A mouse monoclonal antibody, MAb b-12, has been described previously (Stähli et al. 1985) which reacts with a M_r 350 kD glycoprotein with mucin-like characteristics (Stähli et al. 1987) expressed in cytoplasm and on the surface of human breast carcinoma cell lines (MCF-7 and ZR-75-1). In the present report the immunohistochemical reactivity of this MAb with normal and malignant human tissues is analyzed. Pre-experiments showed that the epitope b-12 is resistant to formalin treatment allowing the use of tissue processed by standard paraffin embedding methods. 167 normal and 408 neoplastic tissues were tested by indirect immunofluorescence or the avidin-biotin complex method. MAb b-12 stained the apical cytoplasm of secretory epithelia and their secretions including the acinar and ductular epithelia of the breast. It reacted with all breast carcinomas independent of their histological type or stage, frequently with all but in some cases with a fraction of the tumour cells. Some other carcinomas, primarily those of adenomatous differentiation, were also reactive. In these, however, the fraction of positive tumour cells was usually lower. The b-12 epitope is thus a marker for normal and neoplastic epithelia with secretory functions, particularly for breast carcinomas of all histological types and stages, and perhaps a differentiation marker for abortive adenomatous differentiation in solid carcinomas of the gastro-intestinal, uro-genital or respiratory tract.

Key words: Breast carcinoma – Adenocarcinoma – Tumour cell heterogeneity – Monoclonal antibody b-12 – Tumour marker – Immunohistochemistry

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Introduction

Breast carcinomas continue to be the leading malignant tumour in females in most countries and particular efforts have been made to produce monoclonal antibodies with high tumour specificity, such as Br-1 (Marjani-Constantini et al. 1984), HMFG-1 and 2 (Epenetos et al. 1982), 10-3D2 (Soule et al. 1983), F36/22 (Chogan et al. 1983), Ca1 (Ashall et al. 1982; McGee et al. 1982) and 115D8 recognizing epitope MAM-6 (Zotter et al. 1985). Other epithelial-specific MAb's used for the characterization of breast cancer include cytokeratins (Moll et al. 1983; Nagle et al. 1986), milk fat globule membrane antigens (Hilkens et al. 1981, 1984; Zotter et al. 1985), lactoferrin (Zotter et al. 1985) and CEA (Wahren et al. 1978; Shousha et al. 1978; Kuhajda et al. 1983; Nap et al. 1984; Böcker et al. 1985; Lee et al. 1985). Although none of these was found to be tumour or even organ-specific, some have been found to be useful for histological, serological and in-vitro diagnostic applications when used within a tumour marker panel.

We report here on the immunohistological performance of the monoclonal antibody b-12 which recognizes a tumour associated antigen (Mucinous Carcinoma associated Antigen, MCA: Stähli et al. 1987). It appears to be a tumour marker candidate for both in vitro and in vivo application (Stähli et al. 1987), particularly for the labeling of breast carcinoma, but also for other tumours with secretory differentiation.

Material and methods

The following were analyzed: 82 surgical specimens of primary invasive carcinomas, 30 lymph node and 2 skin metastases and 8 carcinomas in situ. In addition, 167 non-neoplastic and 286 neoplastic specimens from a variety of other organs were se-

lected (Tables 1–4). Immediately after excision, parts of the tissue were snap-frozen and stored in air-tight bags at −70° C. The remainder was fixed in 4% buffered formaldehyde and embedded in paraffin (Paraplast R) according to standard methods.

A detailed description of the generation and biochemical characterization of the MAb b-12 as a IgG_1/kappa mouse monoclonal antibody recognizing a M_r 350 kD glycoprotein (Mucin-like carcinoma associated antigen, MCA) is given elsewhere (Stähli et al. 1985, 1987). Indirect immunofluorescence on unfixed cryostat sections and/or the avidin-biotin-complex (ABC)-method on pronase-treated paraffin sections of formalin-fixed tissues were used as described elsewhere (von Overbeck et al. 1985). The specificity was controlled by replacement of the mAB by phosphate buffered saline (PBS) and parallel incubations of known positive or negative tissues, respectively. The immunohistochemical staining intensity (+−+ + + + +) and the percentage of stained tumour cells (+ = 1–30%, + + = 31–60%, + + + = 61–100%) were graded semi-quantitatively.

Results

In pilot incubations on paired frozen and formalin-fixed, paraffin-embedded sections of 20 breast carcinomas the staining intensity and number of reactive cells were not found to be impaired by the fixation and embedding procedure. Therefore, further examinations were performed with the ABC method on formalin-fixed tissues.

At the cellular level the MAb b-12 stained an intracytoplasmic antigen with a granular pattern. There was a marked concentration gradient of the antigen towards the apical cell pole and the luminal surface. Usually, the content of glandular or ductal lumina was also stained. Cells of neural or mesenchymal origin such as connective tissue, blood vessels, inflammatory cells, smooth and striated muscle were not stained. Table 1 shows the broad distribution of the epitope in epithelia of several organs. In the normal adult breast epithelia of main and terminal ductules, sinus lactiferi and acini showed a strong and even reactivity (Fig. 1). Myoepithelial cells were not stained. In the kidney the b-12 epitope was expressed exclusively in the

Table 1. Reaction of MAb b-12 with epithelia of normal tissues ($n = 167$)[a]

Urogenital tract	
Transitional epithelium	3/3
Kidney[b]	13/13
Ovary	0/1
Fallopian tube	2/2
Uterus	5/5
Vagina	0/1
Prostate	6/9
Testis (Germ-, Leydig cells)	0/6
Epididymis	4/4
Respiratory tract	
Bronchus	13/13
Mesothelium	0/3
Lymphatic tissue	
Lymph node	0/4
Spleen	0/6
Tonsil	0/4
Striated muscle	0/4
Skin	
Stratified epithelium	0/6
Sebaceous and sweat glands	6/6
Gastrointestinal tract	
Salivary glands	3/4
Stomach	6/8
Hepatocytes[c]	0/18
Pancreas excretory	1/4
Endocrine islets of pancreas	0/3
Adrenal cortex	0/6
Thyroid	3/6
Brain (cortex)	0/2
Peripheral nerve	0/1
Myocardium	0/2
Breast	23/23

[a] Number of positive tissue probes / number of investigated specimens
[b] Distal tubules and collecting ducts positive only
[c] Bile duct epithelia positive only

Fig. 1. Normal lobule of the mammary gland showing the apical localization of the epitope b-12 in ductular cells and within the lumen (× 150). Formaldehyde fixation, paraffin-embedded tissue, Avidin-Biotin (ABC-) method, counterstained with haematoxylin

Fig. 2. Immunofluorescent demonstration of epitope b-12 in a medullary carcinoma of the breast. Note diffuse, rather granular cytoplasmic staining with loss of polarization within tumour cells (× 225). Indirect immunofluorescence on acetone fixed, frozen section

Fig. 3. Invasive ductal carcinoma of the breast with intense staining of tumor cells with MAb b-12 (× 150). Formalin-fixed, paraffin-embedded material, ABC-method, counterstained with haematoxylin

Fig. 4. Invasive ductal carcinoma of the breast infiltrating the fibrous tissue. Note antigen-positive globules in singled tumour cells (× 230). Formaldehyde fixation, paraffin-embedded material, ABC-method, counterstained with haematoxylin

Fig. 5. Lymph node metastasis of mammary carcinoma detected in the marginal sinus and afferent lymph vessel by MAb b-12 (× 150). Formaldehyde fixation, paraffin-embedded tissue, ABC-method, counterstained with haematoxylin

Fig. 6. Squamous cell carcinoma of the skin exhibiting focal immunoreaction for the epitope b-12 within the cytoplasm and along the cell membrane of malignant squamous cells (× 230). Formaldehyde fixation, paraffin-embedded tissue, ABC-method, counterstained with haematoxylin

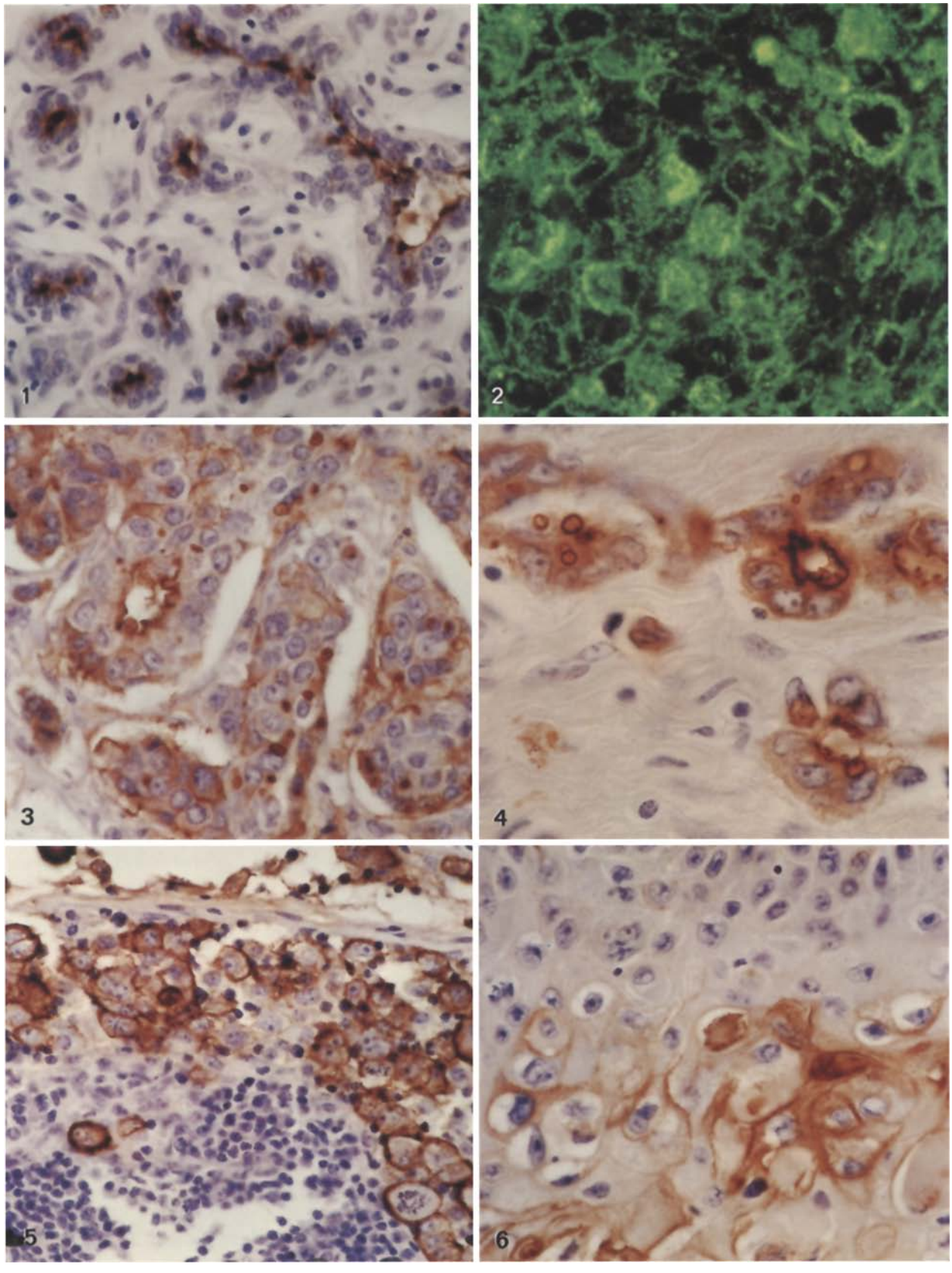
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Table 2. Immunoreactivity of breast carcinoma ($n = 122$) with MAb b-12 and correlation of histological type with grade of epitope expression within the tumour

	Total	High (over 60%)	Intermediate (30–60%)	Low (below 30%)
Ductal carcinoma in situ	5/5[a]	1	2	2
Invasive ductal carcinoma	49/49	22	10	17
Lymph node metastases of invasive ductal carcinoma	25/25	11	6	8
Skin metastases of invasive ductal carcinoma	2/2	0	1	1
Lobular carcinoma in situ	3/3	0	2	1
Lobular carcinoma	18/18	10	6	2
Lymph node metastases of lobular carcinoma	5/5	0	5	0
Medullary carcinoma	12/12	1	5	6
Mucinous carcinoma	3/3	0	1	2
Totals	122/122	45 (36.9%)	38 (31.1%)	39 (32.0%)

[a] Number of positive stained carcinomas / total investigated carcinomas

distal convoluted tubules. Also positive were the transitional epithelia of the renal pelvis, ureter and bladder. The epithelia of the epididymis, prostate, endometrium and Fallopian tube were labeled by the MAb b-12. In contrast, the germ-cells of the testis and ovary were not reactive. In the lung the MAb b-12 reactivity was limited to the respiratory epithelium of bronchioli, bronchi and the bronchial glands. The staining intensity of the bronchial glands was inversely related to the extent of cytoplasmic vacuolization. The respiratory epithelia displayed preferential immunoreactivity in the perinuclear cytoplasm adjacent to the basement membrane. Pneumocytes were not stained. Excretory duct epithelia of salivary glands and the pancreas showed a positive reaction at the luminal cell membrane, whereas acinar and endocrine cells were negative. In the stomach the reactivity was restricted to the bottom of tubular glands which displayed a granular intracytoplasmic staining pattern. In the colon a gradual loss of staining intensity from the bottom to the top of the crypts was observed. In the liver only biliary duct epithelia were weakly labeled. In the skin there was a coarse and vacuolar immunostaining of sebaceous and sweat glands. The epidermis remained unstained. The adrenal cortex and medulla were consistently negative. In the thyroid gland staining of the cytoplasm and particularly of the luminal cell membrane was seen. Labeled follicles were found intermingled with weakly stained or negative areas. There was no difference in the staining intensity between cuboid (presumably active) and flat (presumably inactive) epithelial cells. The colloidal substance in the follicular lumina showed only sparse and weak reactivity.

All 122 breast carcinomas were labelled, however, the overall immunoreactivity was found to be heterogeneous with regard to intensity and fraction of positive cells within a given tumour (Table 2), regardless of the histological type. About 1 third of breast carcinomas showed a generalized expression of the epitope, more than 60% of the tumour area being stained. In 1 third less than 30% of the tumour was labelled and 1 third of tumours were intermediate in staining. The staining patterns of lymph node metastases were identical to those of the respective primary tumour. Mucinous and medullar breast carcinomas were particularly prone to low or intermediate expression. The other histological types, such as invasive ductal and lobular breast carcinoma, showed high grade expression more frequently (Figs. 3, 4). The fraction of positive cells and the staining intensity were not correlated with the grade of differentiation. Premalignant and noninvasive mammary epithelia did not differ from carcinoma cells in staining intensity. There was, however, a striking difference in the staining pattern of poorly versus highly differentiated invasive ductal carcinomas. The former displayed a non-polar, homogeneous, granular intracytoplasmic reactivity whereas the latter showed a strong gradient of expression towards the luminal cell membrane whenever tubules were formed. The loss of polarity was occasionally accompanied by fusion of coarse vesicles into a single vesicle (Fig. 3). Unlike invasive ductal breast carcinomas, lobular carcinomas exhibited a diffuse cytoplasmic pattern. Necrotic areas were generally weakly stained, even when the surrounding vital tumour cells were strongly reactive.

122 of 215 (56.7%) carcinomas of other organs (Table 3), particularly those with adenomatous differentiation, also expressed the b-12 epitope. As

Table 3. Reaction of MAb b-12 with epithelial tumours other than breast carcinomas ($n=215$)[a]

	Total	High (over 60%)	Intermediate (30–60%)	Low (below 30%)
Urogenital				
Uterus				
Endometrium	10/10	6	3	1
Cervix, squamous cell	2/2	–	–	2
Ovary				
Mucinous	4/4	–	2	2
Serous	2/2	–	1	1
Endometrioid	1/1	1		
Mullerian duct	1/1	–	–	1
Thecafibroma	0/1	–	–	–
Granulosa cell	0/1	–	–	–
Testis				
Malignant Teratoma	7/7	–	2	5
Seminoma	0/3	–	–	–
Prostate				
Adenocarcinoma	10/11	–	2	8
Metastatic (bone marrow)	2/5	–	–	2
Kidney				
Clear cell	15/15	3	6	6
Transitional epithelium	5/5	1	2	2
Respiratory				
Larynx				
Squamous cell carcinoma	3/3	–	–	3
Lymphoepithelial	0/1	–	–	–
Lung				
Bronchiolo-alveolar	6/6	2	2	2
Adenosquamous[b]	2/2	–	1	1
Squamous cell carcinoma	3/11	1	1	1
Carcinoid	0/2	–	–	–
Oat-cell	0/1	–	–	–
Mesothelioma[b]	2/2	–	1	1
Gastro-intestinum				
Oral cavity				
Tongue, squamous cell	1/1	–	–	1
Salivary glands				
Cystadenolymphoma[b]	2/2	1	1	–
Pleomorphic adenoma	0/4	–	–	–
Stomach				
Adenocarcinoma	8/9	1	2	5
Liver				
Hepatoma	1/3	–	–	1
Pancreas				
Invasive ductal carcinoma	1/1	–	–	1
Colon				
Adenocarcinoma	24/36	3	3	18
Colon (lymph node)	2/4	–	1	1
Endocrine tumours				
Pituitary adenoma	0/18	–	–	–
Parathyroid adenoma	0/3	–	–	–
Adrenal adenoma	0/7	–	–	–
Adrenal carcinoma	0/7	–	–	–

Table 3 (continued)

	Total	High (over 60%)	Intermediate (30–60%)	Low (below 30%)
Thyroid				
Anaplastic carcinoma	0/3	–	–	–
Follicular carcinoma	0/2	–	–	–
Papillary carcinoma	1/1	–	–	1
Follicular adenoma	1/1	–	1	–
Epidermis				
Squamous cell carcinoma	1/3	–	1	–
Basal cell carcinoma	0/1	–	–	–
Metastases				
Squamous cell carcinoma	2/4	1	–	1
Unknown primaries	2/2	–	–	2
Undifferentiated	1/7	–	1	–
Totals	122/215 (56.7%)	20 (16.4%)	33 (27%)	69 (56.6%)

[a] Number of tumours reacting with MAb b-12 / total investigated specimens
[b] Immunoreaction restricted to the parts of the tumour with adenomatous differentiation

in mammary tumours, there was a heterogeneity in immunoreactivity among tumour cells (Table 3), the majority, however, with intermediate or low extent within the tumour (27% and 56.6%, respectively). Usually, the staining was finely granular, uniformly distributed in the cytoplasm, with a marked staining of the luminal surface (Fig. 5) and a granular immunoreaction of intraluminal material. Unlike breast carcinomas, poorly differentiated adenocarcinomas, regardless of their origin, were found to have lost their ability to express the b-12 epitope. However, squamous cell carcinomas of the tongue, larynx, skin, cervix, and 3 of 11 squamous cell carcinomas of the bronchus showed a cytoplasmic and/or membranous reaction not seen with normal squamous epithelium. Mesotheliomas were labelled only in the tubule forming parts of the tumour. Single tumour cells were only occasionally and weakly positive. With regard to differential diagnosis it should be noted that both mesothelioma and adenocarcinomas, could be labelled by mAB b-12. Among testicular tumours a positive distinction was seen, however, between positively reacting malignant teratoma and negative seminoma. Mesenchymal and neural tumours were consistently negative (Table 4).

Discussion

In the present study we have shown that mAB b-12 recognizes an intracytoplasmic, formaldehyde-resistant epitope synthesized and released by normal and malignant epithelial cells. The granular staining of the cytoplasm, the preferential reactivity of the apical cytoplasm and luminal surface, the positive immunoreaction of secreted products and the reaction with adenocarcinomas suggest that the antigen is released as a secretory product, by analogy with findings with other secreted products such as milk fat globule membrane antigens (Zotter et al. 1985). The partial expression of the epitope b-12 in squamous carcinomas of larynx, bronchus and tongue may be the result of an abortive attempt at anomalous differentiation of these tumour cells towards a secreting cell, a behaviour also seen with CEA (unpublished observations).

Our results show that the b-12 epitope is neither tumour nor organ specific but may be still considered a sensitive marker for breast tumours, since all histological types of noninvasive, invasive and metastatic breast carcinomas are reactive with MAb b-12, regardless of degree of differentiation. In this respect the antibody differs from other, immunohistochemically characterized monoclonal antibodies with some specificity for breast carcinomas (Schlom et al. 1980; Foster C.S. et al. 1982; Horan Hand S. et al. 1983; Imam A. et al. 1985; Kufe W. et al. 1983; Menard S. et al. 1983; Peterson J. et al. 1985; Rasmussen B. et al. 1982, 1985; Iacobelli et al. 1985; White C.A. et al. 1985; Teramoto Y.A. et al. 1982). Of particular diagnostic importance is the consistent reactivity of medullary breast carcinoma – a tumour type which often fails

Table 4. Negative reaction of MAb b-12 mesenchymal and neural tumours

	No. of probes tested and negative for b-12
Lymphomas	22
Non-Hodgkin's lymphoma	17
Hodgkin's lymphoma	4
Plasmocytoma	1
Soft tissue tumours	32
Leiomyosarcoma	9
Leiomyoma (benign)	6
Liposarcoma	2
Histiocytoma	5
Aggressive fibromatosis	2
Hemangiopericytoma	4
Unclassified	4
Neural tumours	14
Astrocytoma	4
Ganglioglioma	1
Meningioma	1
Ependymoma	2
Plexus papilloma	2
Medulloblastoma	2
Neurogenic sarcoma	1
Neurinoma	1
Melanoma	3
Total	71

to react with many other monoclonal antibodies. (Foster C.S. et al. 1982; Schlom J. et al. 1984). This high histological labeling efficiency supports the applicability of serological screening for MCA recognized by mAb b-12 in sera of patients with suspected or proven breast carcinoma (Stähli et al. 1987).

The expression of the b-12 carrying antigen in carcinomas shows features reported for other tumour-associated antigens, e.g. loss of cellular polarity seen in invasive ductal carcinoma in areas lacking ductular differentiation, resulting in condensation of the antigen in coarse vesicles. Another common feature is heterogeneity of immunoreactivity within a given tumour (Imam A. et al. 1985; Teramoto Y.A. et al. 1982; Foster C.S. et al. 1982; Rasmussen et al. 1982, 1985; Kufe et al. 1983; Horan Hand P. et al. 1983; White C.A. et al. 1985; Iacobelli et al. 1985; Schlom J. et al. 1984; Canevari et al. 1983). Edwards et al. (1984) have claimed that phenotypic heterogeneity is not a property restricted to carcinomas but is present in normal ductal epithelia of the breast. For the epitope b-12 we could not confirm this heterogeneity in normal ductular epithelium.

The MAb b-12 consistently reacts with all histological types and differentiation grades of breast carcinomas and with lesser frequency with carcinomas of some other organs. The practical clinical applicability of this antibody, including serological screening, is thus presumably centered on cancers of the breast and highly differentiated adenocarcinomas of other organs such as those of the gastro-intestinal, uro-genital and respiratory tract. Immune light and electronmicroscopic studies are in progress to test the hypothesis that the positivity of squamous cell carcinomas can be taken as a relevant marker for secretory differentiation.

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