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Tumour-associated antigens in mammary and extramammary Paget's disease

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Summary. The immunoperoxidase technique was used to study the immunoreactivities of two murine monoclonal antibodies to carcinoma-associated antigens raised respectively against a human breast cancer line (MBr1) and an ovarian carcinoma (MOv2) and of a conventional anti-CEA serum in 20 cases of mammary Paget's disease of the nipple and in three cases of extramammary Paget's disease. Each of the immunoreagents stained Paget's cells in a high proportion of cases and failed to discriminate mammary from extramammary disease. The antigenic phenotypes of underlying in situ or infiltrating breast carcinomas corresponded to those of the associated Paget's disease of the nipple. The consistent immunoreactivity of eccrine and apocrine sweat glands and of normal mammary epithelia indicated an antigenic relationship between epithelia of adnexal derivation and Paget's cells.

Key words: Paget's disease – Tumour-associated antigens – Monoclonal antibodies – Immunoperoxidase

A number of studies have established the adenocarcinomatous nature of the disease described by Sir James Paget in 1874 (Roth et al. 1977; Azzopardi 1979). Several authors favoured the theory of intraepidermal migration of tumour cells originating in underlying glands such as breast ducts in mammary Paget's disease of the nipple (MPD) (Muir 1935; Inglis 1946; Toker 1961) and adnexal glands in extramammary Paget's disease (EPD) (Weiner 1937; Plachta and Speer 1954; Demopoulos 1970; Belcher 1972; Ferenczy and Richart 1972; Nielson and Woodruff 1972; Lee et al. 1977; Roth et al. 1977). However, the issue of an extra-epidermal (adnexal or mammary) versus an intraepidermal origin could not be conclusively settled, particularly in cases of MPD or EPD not connected to an underlying carci-

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noma (Cheatle and Cutler 1931; Helwig and Graham 1963; Ashikari et al. 1970; Parmley et al. 1975). Developments in immunohistochemistry have recently allowed a functional approach to the study of Paget's disease (Bussolati and Pich 1975; Nadji et al. 1981).

We describe here the immunohistochemical association with Paget's cells of two antigens defined by the murine monoclonal antibodies MBr1 and MOv2 and compare the immunoreactions of these two monoclonal antibodies (MAbs) with those obtained with an anti-CEA serum and with conventional histochemical stains. Antibody MBr1 was raised against the membrane fraction of the human breast cancer cell line MCF7 (Ménard et al. 1983) and was shown to identify a low molecular weight glycolipidic antigen (Canevari et al. 1983). Antibody MOv2 was prepared against the membrane fraction of a mucinous ovarian cystadenocarcinoma (Tagliabue et al., submitted) and identified a glycidic epitope expressed on closely related high molecular weight mucoproteins (Miotti et al. submitted). The immunohistochemical reactivities of the two MAbs were extensively characterised and were confined to neoplastic and normal cells of epithelial lineage (Mariani-Costantini et al. 1984a, b, in press).

Material and methods

Bouin's fixed, paraffin-embedded tissues from 20 cases of MPD (mastectomy specimens) and three cases of EPD (two vulvar, one axillary) were included in this study. An intraductal carcinoma was present in three MPD cases, in 13 cases there was an underlying infiltrating carcinoma, and in four cases MPD was the only neoplastic lesion documented. No cases of EPD were associated with other infiltrating carcinoma. Sections from selected blocks were cut at 5 μ, placed on albumin-coated slides and heated overnight at 37° C. The avidin-biotin-peroxidase complex technique (ABC, Vector Laboratories, Inc., Burlingame, Ca., USA) was used for immunostaining according to described procedures (Hsu et al. 1981; Mariani-Costantini et al. 1984). Diaminobenzidine or 3-amino-9-ethyl-carbazole was used as chromogenic substrate. MAbs MBR1 and MOv2, both of IgM class, were obtained from syngeneic murine ascites, partially purified by ammonium sulfate precipitation and diluted to a final protein concentration of 3 μg/ml (MBr1) and 8 μg/ml (MOv2). Rabbit anti-CEA (Dakopatts, Denmark) was used at a dilution of 1/500.

The following controls were included: the MAbs were replaced by 2% fetal calf serum in Hanks' balanced salt solution or with an unrelated MAb of IgM class normalised to the protein concentrations of MBr1 and MOv2; the anti-CEA serum was replaced by the same antiserum after adsorption with a purified CEA preparation (400 ng/ml) and by normal rabbit serum. Histochemical stains were routinely performed and included periodic acid-Schiff (PAS), Alcian blue, pH 2.5, and high iron diamine-Alcian blue method (HID-Alcian blue) for sulphated and carboxylated sialic acid-containing mucins. The immunohistochemical and histochemical reactions of Paget's cells and associated tumours were scored as: +, i.e., focal staining restricted to individual cells; ++, i.e., staining of most cells. Reactions of adjacent normal histological structures, such as epidermis, epidermal adnexa and mammary epithelia, were searched for and recorded.

Results

Twelve out of 20 cases of MPD tested (60%) contained Paget's cells reactive with MBr1; reactivity was diffuse (Fig. 1a) in six of these cases (30%). The underlying intraductal and invasive carcinoma could be evaluated in

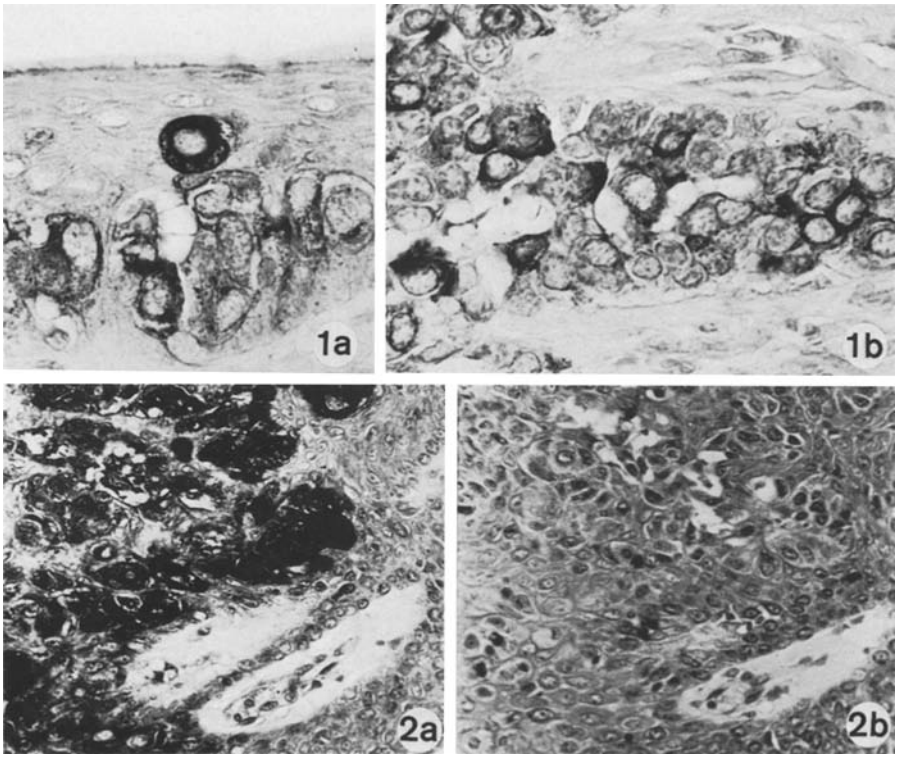

Fig. 1a, b. Similar MBr1 reactions in MPD (**a**) and underlying intraductal carcinoma cells (**b**). (ABC, haematoxylin counterstain, ×1,650)

Fig. 2a, b. Different specificities of MAbs MOv2 and MBr1: MPD cells diffusely MOv2 immunopositive (**a**) are MBr1 immunonegative (**b**). (ABC, haematoxylin counterstain, ×1,030)

16 cases; its immunoreactivity was concordant with that of Paget's cells in 12 of 16 cases (75%; positive concordant, six; negative concordant, six; Fig. 1a and b). One of the three EPD cases tested (from the vulva) was immunoreactive with MBr1; the other two cases were unreactive. Eccrine and apocrine sweat glands in the tissue sections studied were always MBr1 positive, normal mammary epithelia were stained in most of the cases, and a focal membrane reaction of keratinocytes in the malpighian layer was detected in some cases.

Antibody MOv2 reacted positively with 13 of 19 (68%) of MPD cases (one of the 20 cases of Paget's disease could not be evaluated with MOv2 because malignant cells were undetectable in deeper sections). In eight cases (42%), immunoreactivity was diffuse (Fig. 2a). Underlying intraductal or invasive carcinomas were present in 15 cases; immunoreactions concordant with those of Paget's cells were observed in 13 of 15 cases (86%; positive concordant, nine; negative concordant, four). One of the three EPDs (from the vulva) was MOv2 positive. A strong immunopositivity was consistently

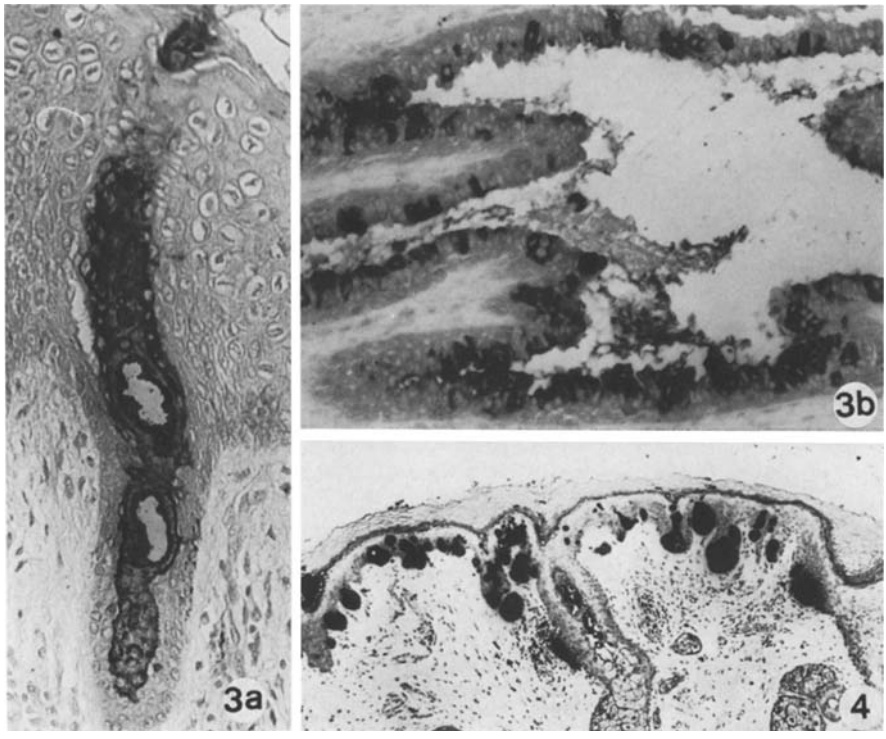

Fig. 3a, b. MOv2 immunostaining of the poral segment of a sweat duct (**a**) and of a normal mammary lactational sinus (**b**). (ABC, haematoxylin counterstain, **a**, ×1,030, **b**, ×660)

Fig. 4. CEA immunoreactivity in MPD. (ABC, haematoxylin counterstain, ×165)

documented in eccrine and apocrine sweat glands, particularly in their poral segment (Fig. 3a), in malpighian keratinocytes adjacent to the poral segments of sweat ducts, and in the intraepidermal portions of collecting mammary ducts. In most cases of MPD, ductal and lobular mammary epithelia reacted positively with the antibody (Fig. 3b).

Immunoreactivity with the anti-CEA serum was documented in 17 of 20 cases (85%); in six cases (30%) labeling was diffuse in most Paget's cells (Fig. 4). Associated infiltrating or intraductal carcinomas were present in 15 cases and their immunoreactions were concordant with those of Paget's cells in 13 cases (87%; positive concordant, 12; negative concordant, one). The three EPD cases were CEA positive. Eccrine and apocrine sweat glands, including their poral portion, were always immunopositive, and normal mammary epithelium had apical labeling in 10 of the 20 MPDs studied. Focal immunostaining of malpighian keratinocytes was occasionally observed.

With regard to histochemical reactions, positive staining of MPD cells was documented in 11 of 20 cases (55%) with PAS and Alcian blue, and

in 12 of 20 cases (60%) with HID-Alcian blue. All three EPDs had Paget's cells positive for the stains used.

There were no correlations between immunohistochemical and histochemical reactions: the cases that scored negative with the PAS, Alcian blue or HID-Alcian blue stains had strong variations in their immunoreactions with MBr1, MOv2 and the anti-CEA serum. Correlations between the immunohistochemical reactions obtained with the different antibodies were attempted in the MPDs tested. The six cases diffusely positive for CEA were either focally reactive (five) or unreactive (one) when tested with MBr1. Conversely, the CEA-negative cases were either strongly immunopositive (two) or immunonegative (one) with MBr1. CEA staining was also unrelated to MOv2: three of these cases were focally immunoreactive, one diffusely immunoreactive and one unreactive with the MAb. CEA-negative cases were either diffusely reactive (two) or unreactive (one) with MOv2. Immunoreactions obtained with MBr1 and MOv2 on the same case were clearly distinct (Fig. 2a and b): of six diffusely MBr1-reactive cases, only two were similarly MOv2 reactive, three were MOv2 negative, and one was focally MOv2 positive. Cases that were MBr1 negative were diffusely reactive (five), focally reactive (one) or unreactive (one) with MOv2. A similar cytological distribution of the labeling was obtained with MBr1, MOv2 and the anti-CEA serum. Immunopositive Paget's cells showed cytoplasmic and circumferential cell membrane reactivity (Figs. 1a and 2a): the staining was similar, irrespective of the cell location at the dermo-epidermal junction or within the epidermis and was clearly distinct from the weaker membrane labeling occasionally observed in malpighian keratinocytes. Strong cell membrane, apical and cytoplasmic labeling were detected in sweat glands, whereas normal mammary epithelia showed mostly apical immunostaining.

Discussion

We characterized some immunohistochemical and histochemical reactivities of 20 cases of MPD and of three cases of EPD, describing the association of two new antigenic phenotypes, defined by the murine MAbs MBr1 and MOv2, with Paget's disease. Immunoreactions with the two antibodies were detected in a high proportion of the cases of MPD tested (MBr1, 60%; MOv2, 68%). A somewhat higher proportion of these cases (85%) was immunopositive for CEA. Neither the two MAbs nor the anti-CEA serum discriminated mammary from extramammary Paget's cells or was specific for malignant cells only in the tissue sections studied. In fact, immunostaining was always documented in eccrine and apocrine sweat glands, including their poral portion, and in normal mammary epithelia. Epidermal keratinocytes were only focally and occasionally immunoreactive. Antibodies MBr1 and MOv2 have been shown to identify glycidic determinants expressed on a glycolipid for MBr1 (Canevari et al. 1983) and on mucoproteins for MOv2 (Miotti et al. submitted). CEA immunoreactivity is also related to glycoprotidic antigens (Harvey et al. 1976). However, we failed to detect

correlations between immunopositivity and positivity with histochemical stains for glycidic components, including acidic glycoproteins (HID-Alcian blue).

Our findings are consistent with the results of previous studies that found antigens shared by the histogenetically related mammary and sweat glands in Paget's cells. MPD and EPD cells were previously shown to react with antihuman casein (Bussolati and Pich 1975) and anti-CEA sera (Nadji et al. 1981). Anticasein reactivity has also been reported in the epithelium of mammary ducts, in intraductal and invasive mammary carcinomas, in sebaceous and sudoriparous glands and in adnexal carcinomas (Bussolati and Pich 1975; Bussolati et al. 1975; Roth et al. 1977). Anti-CEA reactivity has been reported in mammary and extramammary carcinomas associated with Paget's disease (Nadji et al. 1981), in eccrine and apocrine sweat glands, in normal sweat and in benign sweat gland tumours (Nadji et al. 1981; Penneys et al. 1982). In addition, MAbs to human milk fat globule membranes reacted with MPD cells and with mammary epithelium, mammary carcinomas and adnexal glands (Arklje et al. 1981). It would therefore appear that the immunohistochemical phenotype of Paget's cells supports the prevalent theory of Paget's disease as an intraepidermal adenocarcinoma deriving from cells of adnexal origin (Roth et al. 1977; Azzopardi 1979; Nadji et al. 1981). With regard to correlations between the antigenic phenotypes of Paget's cells and of underlying associated carcinoma cells, we found concordant expression of the MBr1, MOv2 and CEA antigens in most MPD cases tested. This strongly suggests that Paget's cells and cells in underlying tumours of the breast are part of the same neoplastic population.

Paget's disease is usually readily diagnosed. However, diagnostic problems may arise in its distinction from malignant melanoma of superficial spreading type (McGovern 1983). Antibodies MBr1 and MOv2 revealed a spectrum of antiepithelial reactivities (Mariani-Costantini et al. 1984a, b, in press; Tagliabue et al. submitted) but were unreactive with malignant melanoma. The detection of the MBr1 and MOv2 antigens may thus be employed as an additional ancillary technique in the discrimination of Paget's cells from malignant melanoma cells.

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