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-

We describe here the immunohistochemical association with Paget's cells of two antigens defined by the murine monoclonal antibodies MBrl 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 MBrl 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 MBRI 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 (MBrl) 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 MBrl 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 MBrl; reactivity was diffuse (Fig. 1a) in six of these cases (30%). The underlying intraductal and invasive carcinoma could be evaluated in