Virchows Arch [Pathol Anat] (1983) 399:355–360

Virchows Archiv A

Case report

Immunoglobulin-A producing probably primary lymphoma of the breast

Per Alm, Lars Brandt, and Håkan Olsson
Departments of Pathology and Oncology, Lund University Hospital, S-221 85 Lund, Sweden

Summary. A breast tumour from a 65-year old woman was found to be a primary non-Hodgkin lymphoma, a very rare primary malignancy in this location. The lymphoma was of a diffuse histiocytic type according to the classification of Rappaport, or polymorphic immunocytoma according to the Kiel classification. Immunohistochemistry, not previously reported for breast lymphomas, revealed the production of IgA. In the serum this appeared as an IgA M-component which was greatly reduced after tumour removal. Immunological properties of primary breast lymphomas are reviewed, we suggest further extended studies with the immunohistochemical use of marker substances for the evaluation of prognosis.

Key words: Primary breast malignant lymphoma – Histopathology – Immunohistochemistry – IgA production

Primary non-Hodgkin lymphomas of the breast are rare and seem to constitute only about 0.1%–0.2% of primary breast malignancies (see Mambo et al. 1977). According to the Rappaport classification most cases reported have been diffuse histiocytic (Wiseman and Liao 1972). To our knowledge no immunohistochemical studies of primary breast lymphomas have been performed.

We report a case with a primary IgA producing non-Hodgkin lymphoma of the breast associated with a peak of monoclonal serum immunoglobulin A.

Case report

A 65-year old woman was admitted to hospital because of a lump in her left breast. She had been healthy until 3 years earlier, when she was treated for recurrent sinuitis. At that time a monoclonal peak of immunoglobulin A (kappa) was found in her serum. The IgA (kappa) peak was estimated to 8.2 g/l. Bone marrow aspiration at that time did not reveal

Offprint requests to: P. Alm at the above address

an increased number of plasma cells. Bence-Jones protein was not found, and there were no other clinical or biochemical signs of myeloma.

Clinical examination and mammography disclosed a tumour strongly suggestive of malignancy and fine needle puncture was suggestive of malignant lymphoma. This diagnosis was finally settled by histopathological examination (see below) after a modified radical mastectomy.

Postoperative recovery was uneventful. The subsequent staging of the lymphoma included pulmonary X-ray, ultrasound investigation of the abdomen, liver and spleen scintigraphy, bone marrow biopsy, fine needle puncture of the liver and the spleen, and blood tests. The patient had no systemic symptoms (fever, night sweats or weight loss). According to the Ann Arbor classification the lymphoma was considered to be stage IE (Carbone et al. 1971).

Blood tests after mastectomy were as follows (normal values given in brackets): Sedimentation rate 19 mm (2–15 mm), haemoglobin 126 g/l (131–163), leucocytes 6.7×10^9/l (4–10), thrombocytes 198×10^9/l (125–400) and serum calcium 2.30 nmol/l (2.20–2.60). Serum electrophoresis disclosed albumin 40 g/l (40–52), Ig-G 13.0 g/l (7.0–15.0) and Ig-A M-component 3.7 g/l (kappa) (8.2 g/l prior to the operation).

Following surgery the patient received radiotherapy with the left axilla and the left chest wall as target areas and to a total dose of 45 Gy in two series; 25 months after the operation and 20 months after the completion of radiotherapy she is healthy and without clinical signs of disseminated disease.

Materials and methods

From blocks of formol-fixed and paraffin-embedded tissue pieces of the surgically resected tissue specimen sections were cut at a thickness of 6 μ. They were then processed for the indirect immunoperoxidase conjugate technique (see Taylor and Burns 1974; Burns 1978) for immunohistochemical demonstration of immunoglobulins (heavy chains – γ, α, μ, δ, ε, and light chains – κ, λ), lysozyme and albumin. The sections were deparaffinized in xylene and brought to alcohol followed by blocking of the endogenous tissue peroxidase activity by a fresh 0.5% solution of hydrogen peroxide in methanol. After washing in phosphate buffered saline (PBS) pH 7.2 the sections were incubated in 5% normal swine serum (NSS)[1] in PBS to reduce unspecific background staining. After removal of excess NSS the sections were incubated with specific rabbit anti-immunoglobulin serum diluted in PBS from $^1/_{200}$ to $^1/_{600}$ (these concentrations were found to be optimal from control experiments using tissues with known reactivity). After washing in PBS the sections were incubated with peroxidase conjugated swine anti-rabbit immunoglobulin diluted $^1/_{20}$ in PBS and further washed in PBS. Staining was then performed through incubation in 0.04% 3-amino-9-ethylcarbazol (Sigma, St. Louis, MA, USA) and 0.01% hydrogen peroxide in 0.05 M acetate/acetic acid buffer pH 5.0. After dehydration and clearing in xylene the sections were mounted in Kaisers glycerin-gelatine (Merck AG, Darmstadt, FRG). To check the specificity of the immunohistochemical staining reaction controls were performed according to Burns (1978). The immunohistochemical staining intensities were subjectively evaluated as weak, moderate or strong compared with control slides. In addition, paraffin sections, cut at a thickness of 6 μ or 1 μ (ultra thin sections), were processed for various light microscopical stains (haematoxylin eosin, van Gieson's connective tissue stain, Gordon-Sweet's reticulin stain, PAS and Giemsa).

Results

Macroscopically, in the lower-lateral quadrant of the left breast there was a tumour measuring $4.5 \times 4 \times 4$ cm. It was located at a distance of about 1 cm from the resection margin of the underneath lying connective tissue fascia of the major pectoral muscle. The tumour was fairly well demarcated

[1] All the immunoglobulin antisera, normal swine serum and peroxidase conjugated swine anti-rabbit immunoglobulin were purchased from DAKO Immunoglobulins AB, Stockholm, Sweden

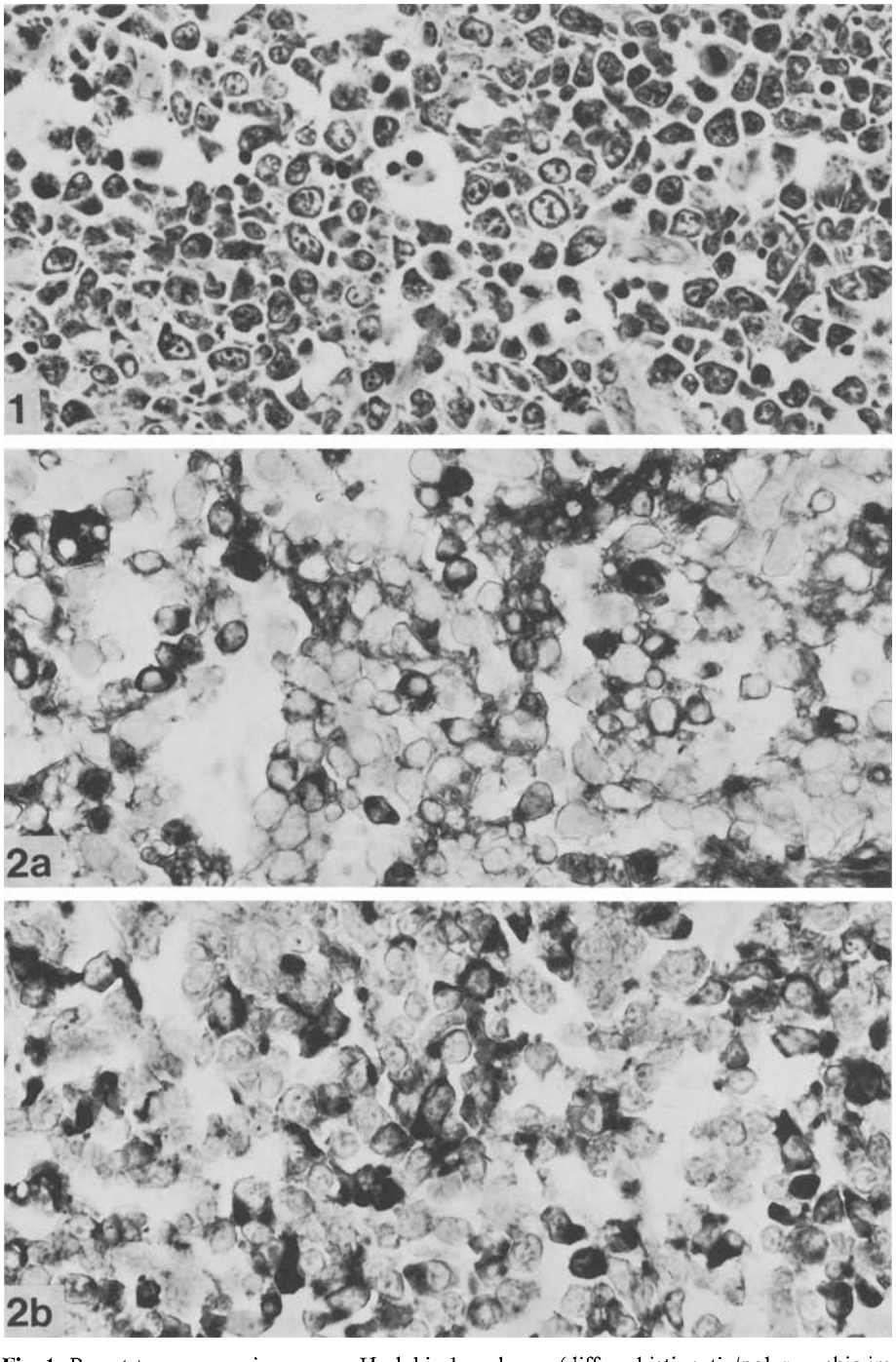

Fig. 1. Breast tumour – primary non-Hodgkin lymphoma (diffuse histiocytic/polymorphic immunocytoma). Giemsa (× 450)

Fig. 2a, b. Sections of the breast tumour showing cells in similar number and distribution with specific immunoreactivities for IgA **a** and kappa chains **b**. Indirect immunoperoxidase technique (× 450 and × 450)

against the surrounding connective and fat tissue. Its cut surface was soft, grayish, and with a lobulated appearance.

Microscopically, the tumour displayed a pronounced cellular and nuclear pleomorphism with many mitoses (Fig. 1). Most cells were large and polygonally or ovally shaped. Their nuclei were large with distinct nucleoli, and they were surrounded by a moderately broad rim of strongly basophilic cytoplasm. The cells correspond to immunoblasts. There were also cells corresponding to centroblasts (medium-sized, with a round to oval nucleus containing several nucleoli located at the nuclear membrane, and surrounded by a narrow rim of strongly basophilic cytoplasm), and centrocytes (small to medium-sized, with a polymorphic nucleus, often with a deep cleft and with several nucleoli, and surrounded by a faintly basophilic cytoplasm). Other tumour cells were generally smaller. They had more or less excentrically located nuclei with varying distribution and density of the chromatin. The broad surrounding rim of cytoplasm was generally strongly basophilic. These cells corresponded to precursors of plasma cells such as plasmablasts and plasmacytoid cells. Only a few mature plasma cells were seen. The tumour cells formed sheets which were partly divided by thin strands of connective tissue from which there emanated delicate reticular fibres forming a diffuse structural network. The cellular distribution pattern was diffuse without follicular or pseudofollicular structures, or structures resembling sinusoids. PAS-positive materials (intranuclear or intracytoplasmic inclusions) and/or amyloid deposits were not found. Toluidine blue or Giemsa staining disclosed only some few mast cells. The histopathological diagnosis was non-Hodgkin lymphoma of a diffuse histiocytic type according to the classification of Rappaport (1966) or a polymorphic immunocytoma according to the Kiel classification (see Lennert et al. 1975).

The immunoperoxidase stainings disclosed in a great number of tumour cells a pronounced reactivity for IgA and κ-chains but not for other immunoglobulins (IgG, IgM, IgD, IgE), lysozyme or λ-chains (Fig. 2a, b). The immunoreactivity appeared as dark-brown precipitates completely covering the cytoplasmic rim around the nucleus. Outside the tumour there were no cells with specific immunoreactivities for IgA or for other immunoglobulins and lysozyme. Extracellularly, IgA immunoreactivity was generally found, with a moderate intensity in the tumour and weak in non-tumour tissue. IgG immunoreactivity, generally with a weak intensity, was also found extra- but never intracellularly. In addition, blood vessel lumina displayed specific immunoreactivities for IgA, IgG and IgM, their respective intensities being strong, moderate and weak. Specific immunoreactivity for albumin, with a moderate to weak intensity, generally occurred extracellularly in all parts of the resected specimen and in blood vessel lumina. Nowhere could intracellular immunoreactivity for albumin be discovered.

Discussion

The patient reported had a malignant lymphoma with an apparent onset in her left breast. The tumour was a diffuse histiocytic lymphoma according

to the Rappaport classification and a polymorphic immunocytoma according to the Kiel classification. In serum a peak of IgA was found but there was no increase of plasma cells in bone marrow, and urine did not contain Bence-Jones protein. Thus, there were no indications that the breast tumour was a manifestation of multiple myeloma.

Immunohistochemical analysis of the tumour cells demonstrated a strong cytoplasmic reactivity for IgA kappa. This reactivity might possibly represent a non-specific uptake of serum IgA. If so the tumour cells might even display immunoreactivities for IgG and/or albumin, particularly as the serum levels of these proteins were higher than that of the IgA M-component (Mason and Biberfeld 1980; Mason et al. 1980). In all parts of the resected breast immunoreactivities for IgG and albumin were found only in blood vessels and extracellularly, but never intracellularly. The results of the immunohistochemical analyses therefore strongly suggest that IgA is produced by the lymphoma cells. This conclusion is further strengthened by the pronounced reduction of the IgA M-component in serum which occurred following removal of the breast lymphoma. In the normal breast, which is a secretory organ, there are many IgA producing plasma cells (Pittard et al. 1979; Hsu et al. 1981). Thus, in view of this it seems reasonable to assume that the present tumour might represent a neoplastic growth from such IgA-producing plasma cells and/or their precursors.

After surgery and radiotherapy there was a residual peak of monoclonal IgA in the patient's serum indicating that lymphoma cells are still present somewhere. There were no clinical or morphological signs of bone marrow infiltration of lymphoma cells but a slight increase in number of such cells in the bone marrow may have been overlooked. In addition to the breast other secretory organs, such as salivary glands and the gastrointestinal tract, are known to attract IgA-bearing lymphocytes (Walker 1976; Weisz-Carrington et al. 1979) and may be the site of IgA-positive malignant lymphomas (Ree et al. 1980). In the present patient residual IgA-producing lymphoma cells may be present in the gastrointestinal tract without causing overt clinical symptoms.

The attraction of IgA-bearing lymphocytes to secretory organs is probably due to the specific microenvironment (Cebra et al. 1977). Further, it has been demonstrated that lymphocytes normally migrate between the female breast and the small intestine (Walker 1976). It therefore seems reasonable that breast tissue might also constitute a favourable environment for IgA-bearing lymphoid cells after a malignant transformation of such cells. It has been reported that 13% of breast lymphomas are bilateral (Schouten et al. 1981) and among 14 patients with primary lymphomas of the breast described by Mambo et al. (1977) 2 had bilateral lesions. This relatively large bilaterality of breast lymphoma might also indicate a homing tendency for certain lymphoma cells in breast tissue and merits further immunohistochemical studies of breast lymphomas in order to characterize these cells.

The survival of patients with primary lymphomas of the breast may vary between a few months and several years (Mambo et al. 1977). The

present patient is in good health about 5 years after the initial demonstration of the monoclonal peak of serum IgA, and apparently the lymphoproliferative disease is not very aggressive. Further extended studies are therefore indicated to elucidate prognostic factors and the immunohistochemical use of various markers may give valuable information for these purposes.

References

Burns J (1978) Immunohistological methods and their application in the routine laboratory. In: Anthony PP, Woolf N (eds) Recent advances in histopathology, vol 10. Churchill Livingstone, Edinburgh, London and New York, pp 337–350

Carbone PP, Kaplan HS, Musshof K, Smithers DW, Tubiana M (1971) Report of the committee on Hodgkin's disease staging. Cancer Res 31:1860

Cebra JJ, Kamat R, Gearhart P, Robertson SM, Tseng J (1977) The secretory IgA system of the gut. Immunology of the gut. Ciba Found Symp 46:25–28

Hsu SM, Raine L, Nayak RN (1981) Medullary carcinoma of breast: An immunohistochemical study of its lymphoid stroma. Cancer 48:1368–1376

Lennert K, Stein H, Kaiserling E (1975) Cytological and functional criteria for the classification of malignant lymphomata. Br J Cancer 31 Suppl II:29–43

Mambo NC, Burke JS, Butler JJ (1977) Primary malignant lymphomas of the breast. Cancer 39:2033–2040

Mason DY, Biberfeld P (1980) Technical aspects of lymphoma immunohistology. J Histochem Cytochem 28:731–745

Mason DY, Bell JI, Christensson B, Biberfeld P (1980) An immunohistological study of human lymphoma. Clin Exp Immunol 40:235–248

Pittard WB (1979) Breast milk immunology. A frontier in infant nutrition. Am J Dis Child 133:83–87

Rappaport H (1966) Tumors of the hematopoetic system. Armed Forces Institute of Pathology, Washington DC

Ree HJ, Rege VB, Knisley RE, Thayer WR, D'Amico RP, Song JY, Crowley JP (1980) Malignant lymphoma of Waldeyer's ring following gastrointestinal lymphoma. Cancer 46:1528–1535

Schouten JT, Weese JL, Carbone PP (1981) Lymphoma of the breast. Ann Surg 194:749–753

Taylor CR, Burns J (1974) The demonstration of plasma cells and other immunoglobulin-containing cells in formalin-fixed, paraffin-embedded tissues using peroxidase-labelled antibody. J Clin Pathol 27:14–20

Walker WA (1976) Host defense mechanism in the gastrointestinal tract. Pediatrics 57:901–916

Weisz-Carrington P, Rouy ME, McWilliams M, Phillips-Quagliata JM, Lamm ME (1979) Organ and isotype distribution of plasma cells producing specific antibody after oral immunization: Evidence for generalized secretory immune system. J Immunol 123:1705–1708

Wiseman C, Liao KT (1972) Primary lymphoma of the breast. Cancer 29:1705–1712

Accepted January 10, 1983