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Monoclonal Antibodies and Human Tumours: Pathological and Clinical Aspects

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INTRODUCTION

FOR MANY years conventional microscopy has been the cornerstone for the diagnosis, classification and prognostic assessment of human neoplasms. However, such appearances, despite their clinical importance, are static pictures of a dynamic situation, seldom yielding reliable information as to those structural and functional properties which could be important in determining the mode of presentation, outcome and therapeutic responsiveness of a particular lesion.

The advent of histochemical, and later immunocytochemical, methods over the past two to three decades heralded a new era in functional pathology. Polyclonal antisera, generally raised to known tissue components, e.g. hormones and enzymes, served as the dominant probes whose location at a cellular level was mostly detected with fluorescent conjugates.

The more recent introduction of peroxidase- or alkaline phosphatase- conjugated antibodies as the endpoint indices has enabled this approach to be extended with reliability to formalin-fixed paraffin-embedded tissues and to the electron microscope level. Good histological detail and accurate localisation can be achieved [1].

The recently developed methodology enabling the generation of monoclonal antibodies, both of rodent [2] and human [3] origin, has provided novel ways to gain new insights into experimental and clinical aspects of oncology. While such reagents to well characterized substances may come to replace conventional antisera, their greatest potential lies in outlining previously unrecognized and uncharacterized cell surface and cytoplasmic components of both normal and neoplastic cells. Such reagents should be of major biological, pathological and clinical value.

Initially, most of the newly derived monoclonal antibodies were directed towards normal and neoplastic lymphoreticular cells [4, 5]. These have proved to be important lineage and differentiation markers while also having therapeutic significance. Now, monoclonal antibodies with relevance to the study of solid tumours are being derived sith similar interesting specificities and potential usefulness.

Many have now been described and are the subject of a recent excellent review [6]. Still others appear in the literature in an almost bewildering profligacy, making workshops on their specificity, molecular basis and potential utility a matter of urgency.

Nonetheless, it is possible to begin to see different areas in which some antibodies may play a role, not least in relation to their utility to analyse in detail, and classify on a more logical basis, various human tumours [7].

SOME PROPERTIES OF IMMUNE PROBES

Tumour specificity

Ever since tumour immunology was conceived, a search for truly tumour specific antigens has been conducted. Despite the resolving power of the monoclonal technology, no such antigen has as yet been described in convincing terms (see review by Lloyd [6]).

Differentiation analysis

Differentiation is associated with progressive cellular maturation with acquisition of different structural and functional phenotypes. Monoclonal antibody probes are beginning to be found to be valuable reagents to chart such processes.

Lineage specificity. Monoclonal and polyclonal probes have been derived with specificity for the different lineages to which cells belongs e.g. germ cell layers. These antibodies are directed principally towards various cytoskeletal proteins [8] such as the intermediate filaments, which comprise five major groups: keratin, vimentin, desmin, neurofilaments and the glial fibrillary

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acidic protein (GFAP). Each class shows a strong preponderance to be restricted to a particular cell lineage: keratin to epithelial cells (Fig. 1), vimentin to mesenchymal cells, desmin to muscle, neurofilaments to neural cells and GFAP to the glia. Nonetheless, some exceptions have been described [8]. Application of such antibodies has already proved important in tumour diagnosis, where in general the immunological characteristics of tumour cell intermediate filaments are those present in their tissue of origin [9–11].

Hence it is possible to assign an undifferentiated small cell tumour to either epithelial, mesenchymal or lymphoreticular origin, a fact of therapeutic value.

Cell and tissue specificity. During embryogenesis cells show a propensity to evolve and develop along different lines to form particular cellular subsets and eventually organs. Interesting cell surface changes have been described during these processes [12]. In the adult, however, until recently, the best examples of cell- and tissue-specific markers were hormones, enzymes and their isoenzymic forms. Through the use of monoclonal antibodies, interesting differences in the cell surface compositions of different tissues and cell types therein are being recognised. To date, reagents are available to delineate uniquely various cell types such as lymphoreticular cells, melanocytes, renal or prostatic cells (see [6]). In many other instances, monoclonal antibodies may outline cell surface properties shared by several, but not all, tissues. Some of these may be 'housekeeping', i.e. common antigens or antigenic epitopes.

During the early stages of development, cytokeratins appear to be the first intermediate filaments to be expressed and in the adult 19 different cytokeratins have been identified and catalogued in human epithelial tissues using two-dimensional gels [13]. From these studies it has been demonstrated that different epithelia contain specific combinations of the subsets of cytokeratins. Thus by using combinations of monoclonals specific for them it may be possible to further subdivide carcinomas and to identify the tissue of origin by immunological techniques [14].

Maturation antigens

The vast majority of antigens presently delineated by monoclonal antibodies are directed towards cell surface epitopes and are best regarded as maturation antigens, i.e. detecting a state of development or evolution in the construction of a particular cell lineage [1]. The simplest examples, long recognized, are the surface immunoglobulins of B cells, plasma cells and their tumours. A variety of monoclonal antibodies have been described which are T cell-related and outline their structural and functional differentiation status both in normal situations and in neoplasia [15, 16].

Further examples with relevance to solid neoplasms include monoclonal probes to melanocytes, astrocytes and various kidney cell types. Such reagents appear to depict different degrees or routes of differentiation. For example, some monoclonals only outline glomeruli, others the proximal tubules, while still others the distal tubules. Hypernephromas possess the epitopes of proximal convoluted tubules. The current use of antibodies to melanocytes and astrocytes and their utility in relation to their corresponding tumours has recently been reviewed [5].

Antibodies to specific cytokeratins also clearly delineate populations of cells within a specific epithelial cell type as demonstrated by the staining of the basal layer of human epidermis in Fig. 2 [17]. This is in contrast to the staining of all layers with a polyclonal antiserum raised to a total keratin extract of human callus (Fig. 1). Such markers are now providing the tools to dissect the histogenesis of skin tumours [18, 19] and perturbations of the normal staining pattern in various skin conditons are indicators of the defects of cellular maturation occurring in conditions such as eczema and psoriasis [20].

IMMUNE PROBES AND BREAST CANCER

It is not possible within this short account to review the state of the art with respect to every human tumour. Accordingly, this presentation will be directed to some of the immunological probes which are of potential pathological and clinical value in the analysis of the human breast and its tumours.

Lineage specificity

The normal human breast duct and lobuloalveolar system consists of two dominant cell types—epithelial cells, which line the lumina, and myoepithelial cells, which form a peripheral ring of cells around the ducts and which abut onto the outer limiting basement membrane. The ectodermal origin of the breast ductal system is reflected by the intermediate filament protein expression by normal breast cells, with keratin being present in both the epithelial and myoepithelial cells [21].

Cell and tissue specificity

Several immunological probes have become available which delineate the different cell types of the resting normal breast. Myosin is preferentially expressed cytoplasmically by myo-

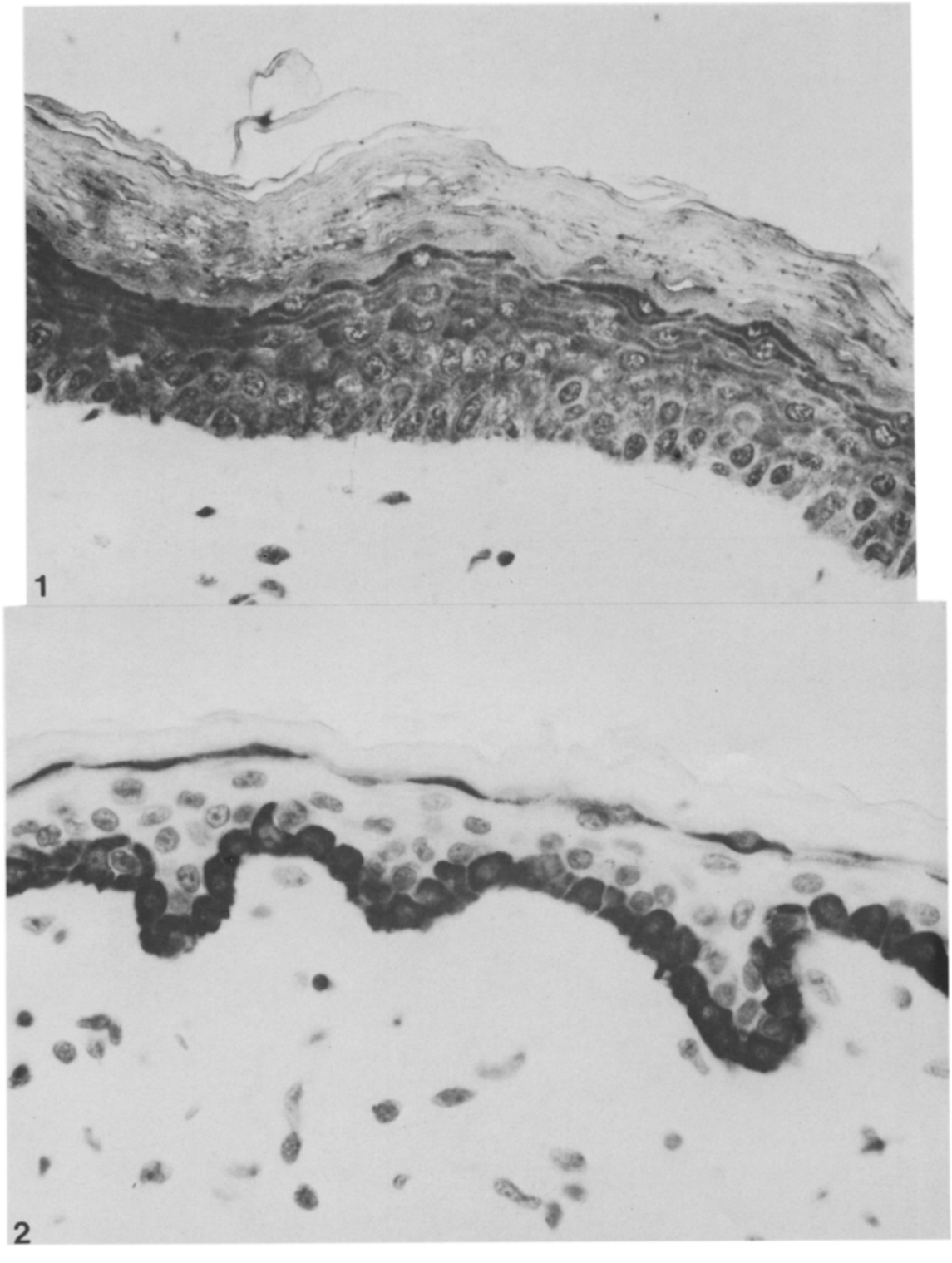


Fig. 1. Skin from the chest wall stained (immunoalkaline phosphatase) with a polyclonal rabbit antiserum raised to a total keratin extract of human callus. There is staining of all layers. ×500.

Fig. 2. Skin from the chest wall is stained using the indirect alkaline phosphatase technique with a mouse monoclonal antibody which recognises cytokeratins of molecular weight 45k *and* 46k. *There is intense staining in the granular layers due to the presence of numerous basophilic keratohyalin granules.* ×500.

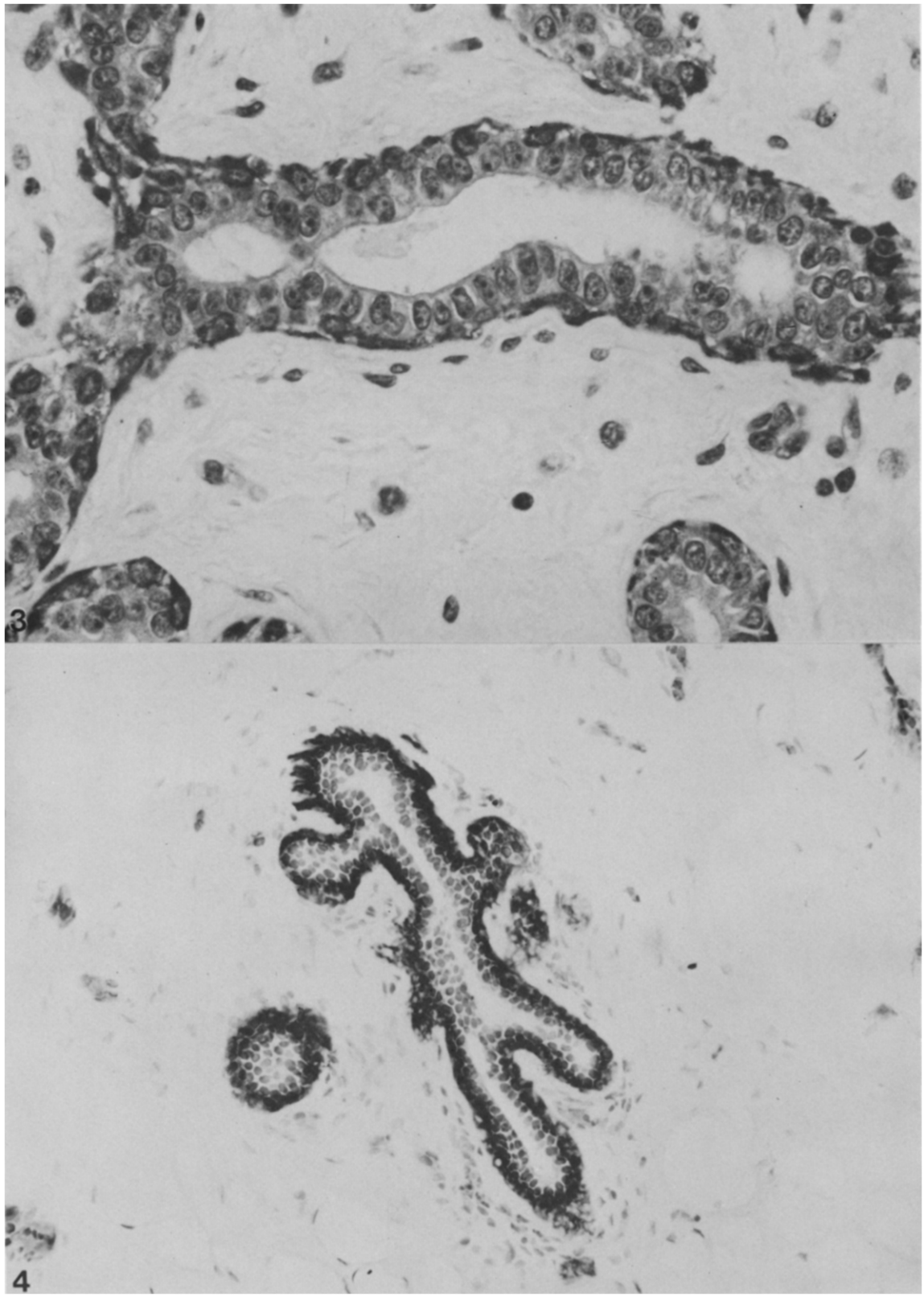
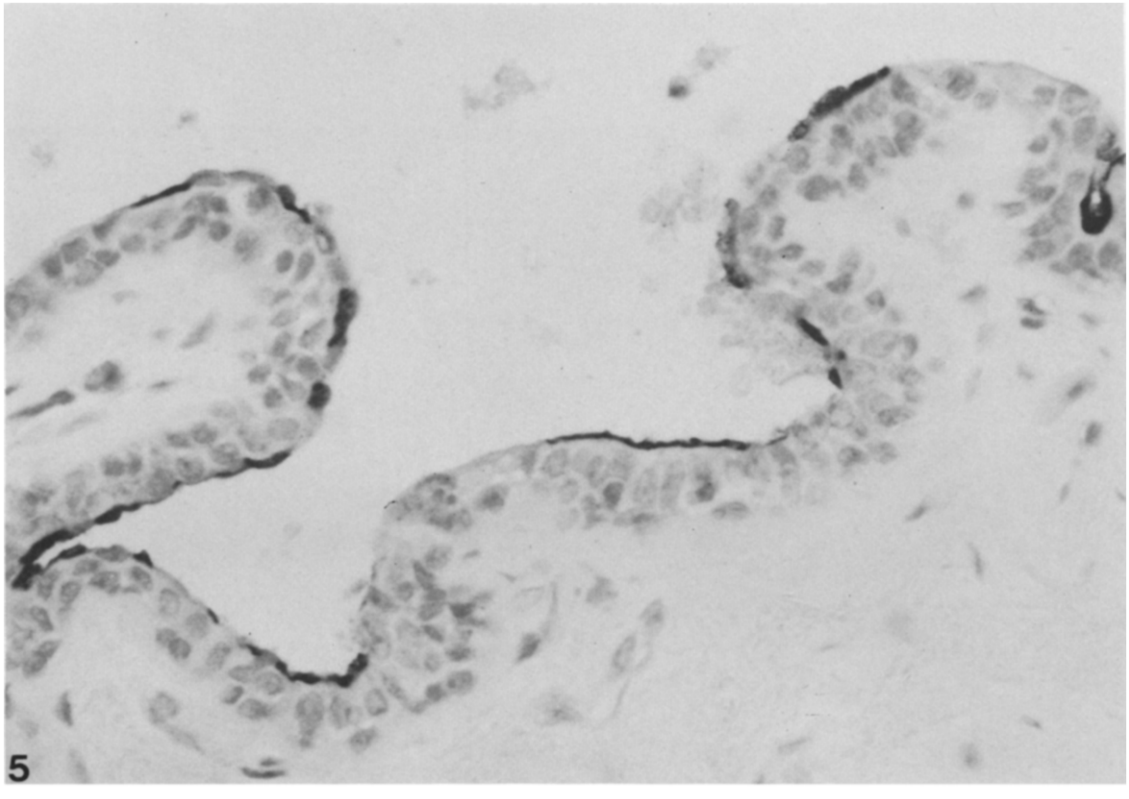


Fig. 3. Normal human breast duct. An immunoalkaline phosphatase method using antibodies to myosin shows the peripheral myoepithelial cells and their content of this constituent. ×500.

Fig. 4. Normal human breast duct. An immunoalkaline phosphatase method using the LICR-LON-23.10 monoclonal antibody outlines the cell membranes of the myoepithelial cell population. ×250.



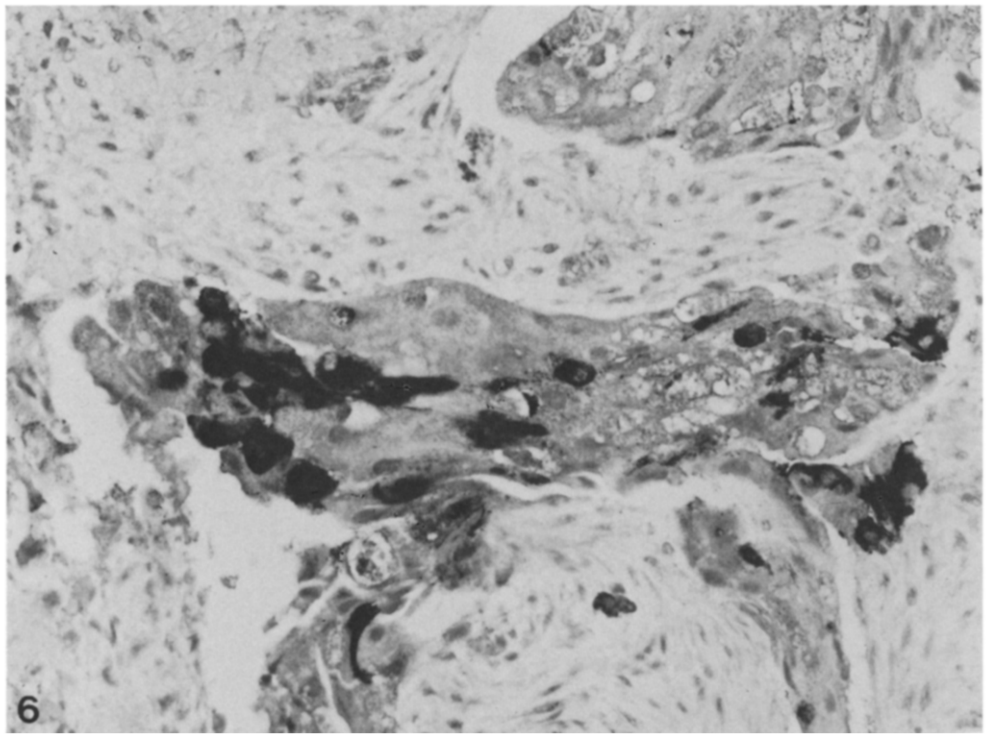


Fig. 5. Human breast duct. A normal breast duct has been immunostained to show the distribution of a monoclonal antibody, LICR-LON-M8. Note the heterogeneity of expression of the epitope. ×300.

Fig. 6. Human breast carcinoma. An immunoalkaline phosphatase tehnique with the monoclonal antibody, LICR-LON-E_{36}, highlights those tumour cells expressing neuroendocrine differentiation. ×350.

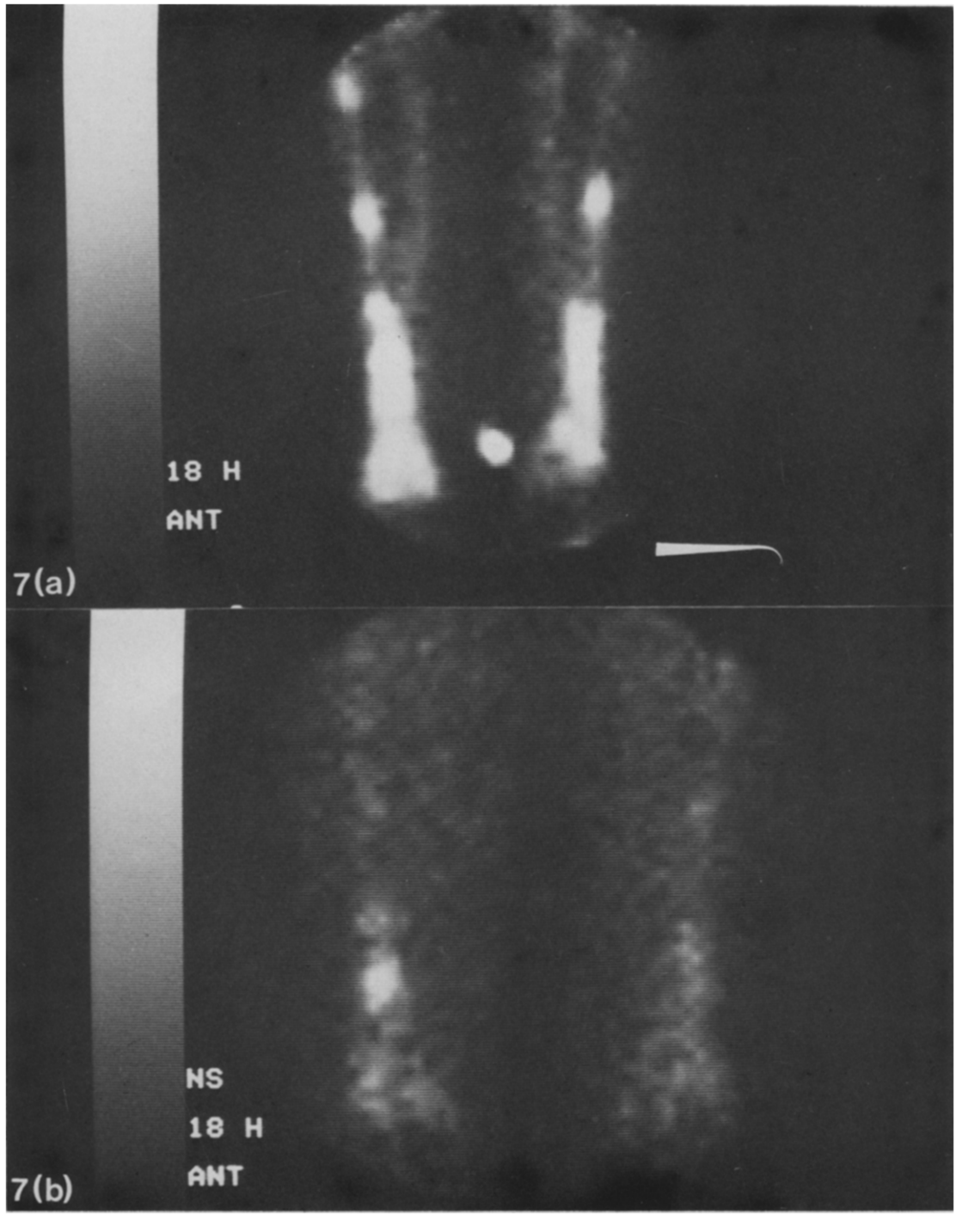
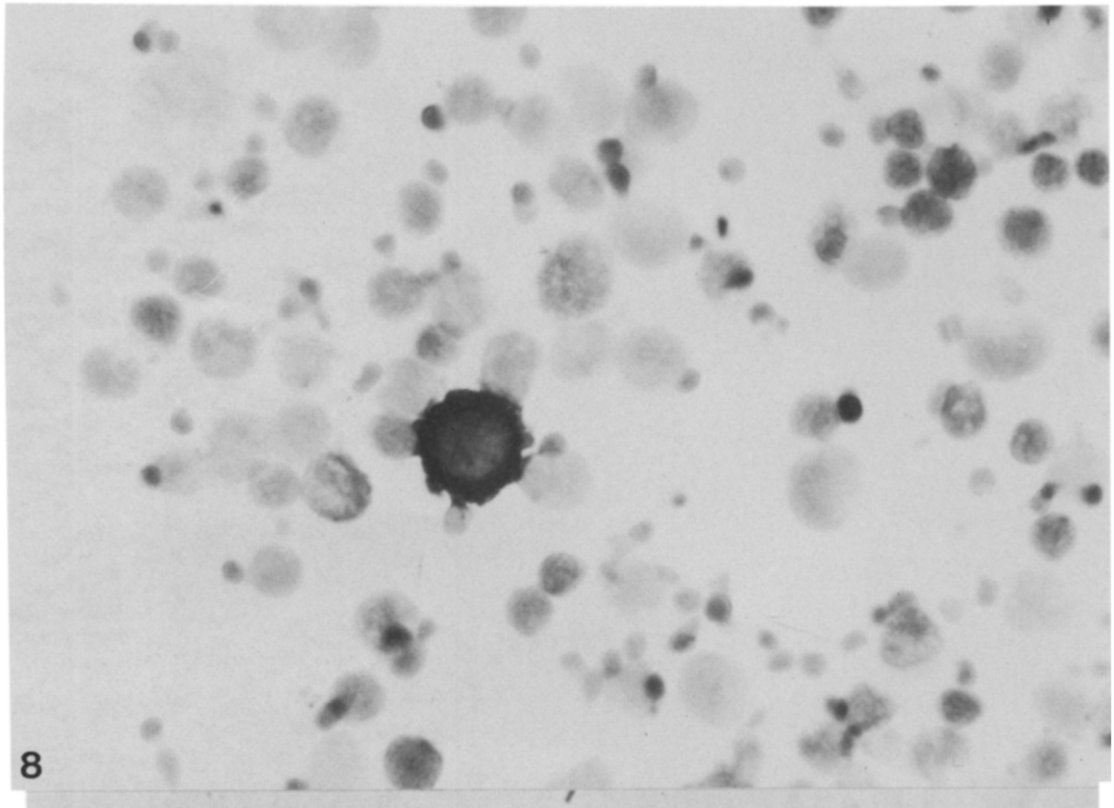


Fig. 7. Immunoscanning using monoclonal antibodies. 18-hr images of the femoral metastases are depicted: (a) [^{111}In]DPTA-M8 image; (b) [^{111}In]DPTA-IgG$_1$ (control) image.



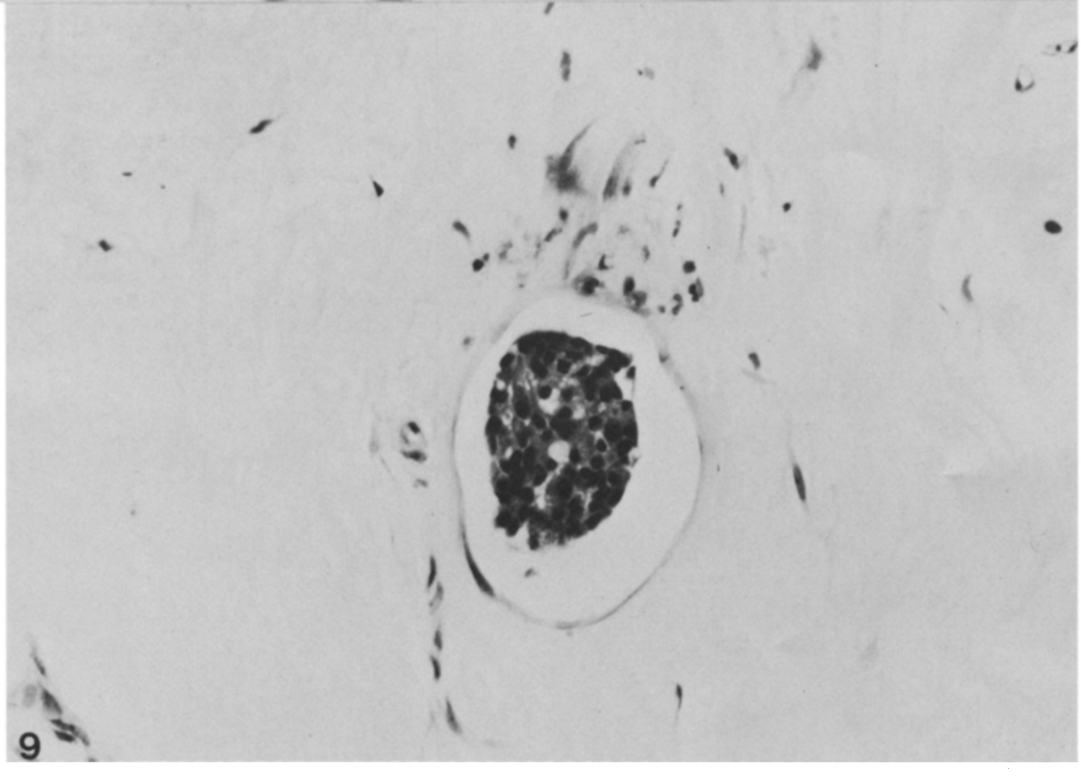


Fig. 8. Bone marrow smear. An immunoalkaline phosphatase preparation using polyclonal antibodies to the epithelial membrane antigen (EMA) to outline a breast cancer cell (centre). None of the marrow cells is stained. ×240.

Fig. 9. Human breast cancer. A small vascular channel in close proximity to the carcinoma contains tumour. $H + E$, ×250.

epithelial cells (Fig. 3) [21]. A monoclonal antibody, LICR-LON-23.10, detects a cell surface component preferentially possessed by myoepithelial cells (Fig. 4) [Gusterson and McIlhinney, personal communication]. The use of monoclonal antibodies (*vide supra*) to the keratins of breast epithelial cells reveals that different keratins may be expressed by morphologically identical epithelial cells, a phenomenon which may also be relevant with respect to the structural or functional maturation of such cells.

Maturation antigens

Several groups of workers [22-28] have derived a series of polyclonal and monoclonal antibodies to the human milk fat globule membrane (MFGM). Such antibodies mainly detect carbohydrate epitopes of both cell surface glycoprotein and glycolipid components possessed only by the epithelial cells (Fig. 5). Such antigens are best regarded as maturation moieties reflecting different functional states. While other functional properties of the epithelial cells in the resting state remain unknown, during lactation, these cells produce milk proteins. Experimental studies have shown that the normal differentiated myoepithelial cells are the dominant source of basement membrane components such as laminin and collagen IV [29, 30].

Changes in breast disease including carcinomas

In benign breast diseases the basement membrane can be shown to be intact with myoepithelial cells surrounding such lesions. With the evolution of *in situ* carcinomas, while the basement membrane still forms a continuum, the myoepithelial cells become attenuated and fewer in number. With the evolution of foci of invasion, the basement membrane becomes defective and myoepithelial cells, i.e. fully differentiated myosin or 23.10 positive cells, are no longer demonstrable. Epithelial-directed antibodies continue to stain such carcinoma cells [21].

Such a change may be an important factor in the invasive process, playing a role *pari passu* with degradative enzyme systems [31]. Nonetheless, this is not an all or nothing phenomenon in that, in some well differentiated cancers, basement membrane or myoepithelial cells may be demonstrable focally. Rarely an invasive tumour with the appearance of a 'lobular carcinoma' may be found when myoepithelial cells appear to be the dominant cell type.

Other monoclonal antibodies, directed principally towards cytoplasmic constituents, may give some indication of the degree of differentiation of a particular breast carcinoma. These include probes for the estrogen receptor, of therapeutic and prognostic significance [32], a secretory component, a constituent whose synthesis may be controlled by estrogen [Gore, personal communication], placental proteins [33] and estrogen-metabolizing enzymes [34-35]. Dispute exists as to whether breast cancers synthesize milk proteins; molecular biological studies would tend to negate such a differentiation phenomenon.

From time to time carcinoid tumours have been recorded as being of breast origin [36]. Their site of evolution in the breast has been somewhat of a mystery. Recently a monoclonal antibody, LICR-LON-E_{36}, has delineated a small number of cells in the normal duct as containing neurosecretory-type granules [Monaghan *et al.*, personal communication]. Such expression has been noted in ~30% of invasive duct cancers (Fig. 6).

The use of cell surface directed monoclonal antibodies has revealed heterogeneity of expression of the antigens or epitopes (Fig. 5) being detected in almost all the systems studied to date. This is a property of normal cells with apparently similar morphological and ultrastructural appearances and is also manifested by their corresponding tumours [37]. In the breast this phenomenon is not cell cycle-related and following single normal cell cloning, epitopes not initially expressed may subsequently arise. While the molecular nature of the phenomenon needs to be determined in detail, it may represent a differentiation response to particular environmental stimuli.

Nonetheless, certain monoclonal antibodies can delineate most of the cell population. In the breast LICR-LON-M8 is such a reagent [37], marking most luminal cells, and all or almost all cells in isolated situations. This enables this probe to have potential utility for the clinical detection of neoplasms.

CLINICAL APPLICATIONS

Tumour detection

Many studies have been effected in an attempt to evolve better methods for the detection of metastatic disease. Biochemical indices measured in the blood or urine have proved to be successful for tumours of germ cell, colonic and prostatic origin and lymphoreticular diseases [38]. Similar approaches in breast cancer have delineated several marker substances whose levels are raised in the blood and/or urine in overt metastatic disease, e.g. plasma carcinoembryonic antigen (CEA), alkaline phosphatase, γ-glutamyl transpeptidase and urinary hydroxyproline [38]. However, when sequential studies are conducted the lead time between becoming biochemically malignant (i.e. pathologically elevated levels) and

the detection of metastases by other clinical and radiographic methods is short, of the order of 3-4 months [39, 40]. Consequently, various workers have sought alternative methods.

Radioimmunolocalisation. Using polyclonal antibodies labelled with different isotopes of iodine, several groups have demonstrated that external scanning procedures can localize both primary and metastatic tumours. Clinically, this approach can detect lesions not identified by other conventional radiological, nuclear scanning or ultrasonographic methods [41-44]. In these studies antibodies to the carcinoembryonic antigen (CEA), alphafetoprotein (AFP) and βHCG have been employed [41-45] Evidence has been gathered to suggest that, in some instances, such antibodies may not localize on the tumour cells but in areas of secretion of these moieties within the tumour itself [46].

Accordingly, monoclonal antibodies directed to tumour cell surface components may offer an advantage. Monoclonal antibodies to CEA have been studied in detail, although others to melanomas, glial and colonic tumours have been employed [47-51]. The use of F(ab) fragments can yield even more striking results [52]. However, access (i.e. vascularity) to tumours remains a problem, as does heterogeneity of antigen expression. Doubts have also been expressed regarding the utility of iodine as a suitable scanning isotope.

A new approach has been achieved through the conjugation of monoclonal antibodies to bifunctional chelating agents and, then, labelling them with more suitable scanning isotopes [53]. Our own experience in this context is concerned with the use of the breast-directed monoclonal antibody, LICR-LON-M8, conjugated with DTPA and then labelled with Indium (^{111}In). This reagent yielded superior results in model xenograft systems [5].

Patients with known breast cancer metastases were then studied and the utility of immunoscans compared with conventional radiography and MDP bone scans. The immunoscan could detect disease not found by either of the other methods but also *vice versa* (Table 1) (Fig. 7). These differences, in part site-dependent, may also be related to the stage of evolution of the metastases [53]. MDP is taken up into the interphase between osteoid and mineralised bone, at sites of new bone formation, so that a positive MDP scan may reflect a response to bone destruction by an expanding metastasis. The uptake of the immunoreagent should be independent of this process and may occur before new bone lysis or sclerosis has taken place, i.e. at an early stage of medullary invasion. Further studies are needed to corroborate this.

Immunocytochemistry and micrometastases. Radioimmunolocalisation still detects breast metastases too late in their evolution. Most patients at primary presentation do not have overt evidence of metastases as measured by conventional techniques [54, 55]. Nonetheless, 50% will develop metastases within 5 yr. Of these 57% will be found to have bone metastases at first relapse [5]. One approach could be based on the immunocytochemical use of anti-breast probes to detect such tumour cells in marrow samples at the time of first presentation.

Conventional cytology can detect tumour cells in the marrow of patients without overt bone metastases but in only a minority (~1%) of patients. Using a polyclonal probe to human breast cell membranes (anti-EMA), we found that

*Table 1. Analysis of differences between M8 images, MDP images and X-rays**

Region of interest	Skull	C/spine	D/spine	L/spine	Ribs	Pelvis	Femora	Humeri
No. of patients studied	7	7	9	5	9	13	9	5
No. of lesions detected (M8 + MDP + X-ray)	16	6	17	14	11	41	33	13
No. of hot spots on M8 images	11	6	7	8	2	27	30	11
No. of M8 hot spots not correlating with MDP (%)	6 (55)	3 (50)	1 (14)	6 (75)	1 (50)	14 (50)	11 (37)	2 (18)
No. of M8 hot spots not correlating with X-ray (%)	6 (55)	5 (83)	0 (0)	3 (38)	2 (100)	8 (30)	13 (43)	3 (27)
No. of lesions not detected on M8 images (%)	5 (31)	0 (0)	10 (59)	6 (43)	9 (82)	14 (34)	3 (9)	2 (15)

*From Rainsbury *et al.* [53].

immunocytochemical methods applied to such smears could increase the detection rate in patients with recurrent non-skeletal disease. Such tumour cells occurred not only in small clumps but also as single cells, i.e. micrometastases (Fig. 8). These patients subsequently pursued a worse prognosis, with earlier development of overt bone metastases than those in whom tumour cells were demonstrable in the marrow [50].

Accordingly, this study was extended to marrow smears harvested from 200 patients at the time of their initial presentation and in whom there was no evidence of metastases as determined by detailed clinical, biochemical and radiographic methodologies. The incidence and number of tumour cells, i.e. 'micrometastases', detected by immunocytochemistry are shown in Tables 2 and 3 [57].

The presence of intratumoural vascular and/or lymphatic permeation has been found to be an important prognostic variable (Fig. 9) [58]. The incidence of marrow micrometastases appears to correlate well with it and other known prognostic indices (Table 4). Of greatest importance may be the detection of micrometastases in a small subgroup of patients with so-called 'good prognosis' disease (i.e. VI-, N-, ER+; see Table 4). They may benefit from active systemic therapy.

Present methods are not detecting all the patients who subsequently develop bone metastases. Other immune probes such as to cytoplasmic keratins and/or a cocktail of cell surface directed monoclonal antibodies need to be examined for their accuracy of detection. Appropriate immune probes are also currently available to detect early marrow involvement by other types of tumours such as oat cell, prostatic, thyroid, renal and colonic carcinomas and neuroblastomas.

*Table 2. Incidence and number of tumour cells detected in 110 consecutive marrows from patients with breast cancer at first presentation**

No. of tumour cells detected	No. of patients
0	79
1-20	9
21-40	7
41-60	7
61-80	3
>80	5
Total	110

*Data of Redding *et al.* [57].

Table 3. Incidence of tumour cells in the marrow as a function of nodal status

Axillary nodal status	Tumour cells in the marrow detected by immunochemistry		
	Present	Absent	Total
Positive*	29 (33%)	58	87
Negative	23 (20%)	90	113
Total	52 (26%)	148	200

*One or more nodes involved.

Prospects for therapy

It has long been hoped that antibodies directed towards tumour cell surfaces could be employed as therapeutic agents. This hope has remained elusive until the advent of monoclonal antibodies, when new efforts were made in this direction. While the use of the antibodies themselves has been considered and put into practice [59], more attention tends to have been paid to the possibility of using them as homing agents carrying cytotoxic agents or isotopes. In this context, the plant toxins, ricin and abrin and Pokeweed Antiviral Protein (PAP) have been given most consideration [60, 61].

Systemic approaches. Unconjugated monoclonal antibodies have been used to treat patients with leukaemias, lymphomas, colonic cancer and melanomas [59]. In general, remissions were not obtained although some success was claimed in relation to T cell lymphomas. One outstanding exception has been the use of an anti-idiotypic antibody in a patient with a B cell lymphoma who 15 months later was still in remission [62].

There are numerous problems with these forms of therapy, including tumour cell antigenic modulation and anti-mouse antibody generation by the patients. With respect to solid tumours, there is also a problem of antibody penetration which may be vascular-dependent but could potentially be overcome by repeated doses of antibody if each dose induced some necrosis. In addition, as mentioned before, antigenic heterogeneity to most monoclonals is often apparent so that several monoclonals may need to be used simultaneously or sequentially. Nonetheless, in model xenograft breast cancer systems the use of two monoclonal antibodies, both complement-

Table 4. Incidence of micrometastases as a function of several prognostic indices

Prognostic variable*	% patients, tumour cells in the marrow	
	Present	Absent
VI+ ER-, N+	82	18
VI+, ER-, N-	75	25
VI-, ER+, N+	33	66
VI, ER+, N-	12	88

*VI, intratumoral vascular and/or lymphatic invasion; ER, estrogen receptor status; N, axillary nodal status.

dependent, has resulted in tumour cell necrosis, the effects lasting for 4-30 days after single doses. Regrowth of the tumours then took place [63].

Bone marrow cleansing. Because many monoclonal antibodies exhibit specificities for more than one tumour or tissue type, their *in vivo* use is precluded. Thus attention has now been directed towards their role *in vitro*, where they can be wholly specific for particular tumour cell types. In this way, they may have a role to play in cleansing the bone marrow of tumour cells.

Bone marrow toxicity represents a limiting feature in many effective therapies for leukaemias and lymphomas. High-dose therapy with similar side effects is now also under consideration for other tumours, such as neuroblastoma and breast cancer. Hence the possibility of eliminating tumour cells from the marrow, its cryopreservation during the high dose regimen and then use to reconstitute haematopoeisis is of paramount interest.

Several current trials of ALL employ monoclonal antibodies to cleanse the marrow for subsequent autologous transplantation [59]. Successful haematopoeisis can be reconstituted; the long-term results are keenly awaited. A monoclonal antibody recognising breast cancer but not marrow progenitor cells has been shown to be effective with complement, in killing such tumour cells without damage to the bone marrow [64]. Coupling of the antibody to the A chain of ricin or abrin creates a more effective agent in model *in vitro* systems. Clinical trials are now underway.

Similarly anti-neuroblastoma antibodies, bound to polystyrene microspheres containing magnetite, have been shown to remove such tumour cells from the marrow [65].

The successful use of monoclonal antibodies in association with complement or conjugated to ricin to rid the marrow of T cells is paving the way to the use of mismatched marrow for post-high-dose engraftment [66, 67]. This approach also has interesting possibilities for certain autoimmune disorders [68].

CONCLUSIONS

The variety of monoclonal antibody probes which are now available can herald a new era in experimental and clinical oncology. Much remains to be achieved at a biochemical level to characterize the epitopes and antigens with which they interact and their functional significance. Nonetheless, they are paving the way to improved pathological evaluation of tumours while also giving new reagents to assist in tumour detection and treatment.

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