

REACTION OF MONOCLONAL ANTI LEU M1— A MYELOMONOCYTIC MARKER (CD15)— WITH NORMAL AND NEOPLASTIC EPITHELIA

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SUMMARY

The present study describes the distribution of Leu M1 (CD15) immunoreactivity in 13 normal epithelia and 14 epithelial neoplasms. The findings are compared with those obtained using other epithelial markers. Cautionary points and broad recommendations are made with regard to use of anti Leu M1 antibody.

KEY WORDS—Leu M1, epithelial neoplasia, myelomonocytic antigens

INTRODUCTION

A monoclonal antibody raised against a human monocyte cell line (U937) and designated anti Leu M1 is known to identify a differentiation antigen (CD15) on human myelomonocytic cells,¹ and to recognize determinants of Reed–Sternberg cells of most variants of Hodgkin's disease (HD).^{2,3}

The CD15 antigen has recently also been detected on and within neoplastic cells of several forms of T cell lymphoma/leukaemia^{4,5} and on *in vitro* mitogen-stimulated, normal T helper/inducer lymphocytes.¹

During the course of our immunohistological investigations of extra-nodal lymphoma, we noted consistent, specific staining of structures such as the salivary gland and gastrointestinal epithelial cells by the anti Leu M1 antibody. We have now performed a systematic study of various normal and malignant epithelia, our aim being to document the immunoreactivity of anti Leu M1, compared with other well characterized monoclonal antibodies with specificity for carcinoma and some normal epithelia. The antibodies used for comparison were to carcinoembryonic antigen (CEA), to intermediate filament cytokeratins (Cytok) to epithelial membrane antigen (EMA)^{6,7} and antibody to a carbohydrate antigen designated CA19-9.⁸

MATERIALS AND METHODS

Tissues for investigation were obtained from files of the Aberdeen Pathology Department. All tissue had been routinely fixed in 10 per cent neutral buffered formalin and embedded in paraffin wax. The histopathological diagnoses in all cases were checked by one of us (WDT).

Immunohistochemistry

5 micron, dewaxed sections were trypsinized for 30 min at room temperature (RT) and endogenous peroxidase inhibited using a methanol/hydrogen peroxide mixture. The buffer used throughout for washings and antibody dilutions was 0.05M Tris-HCl buffered saline (TBS), pH 7.6.

A three stage immunoperoxidase method was used. The sources, specificities and dilutions of the primary layer monoclonal antibodies and the secondary and tertiary layer peroxidase labelled polyclonal antibodies are shown in Table I.

The mouse monoclonals were applied to TBS washed sections for 60 min at RT. After washing (3 × 2 min changes) the second layer peroxidase labelled rabbit anti mouse Ig was applied for 30 min. Following washing in TBS, the tertiary layer swine anti rabbit Ig peroxidase labelled antibody was applied for 30 min, the bound peroxidase was visualized using the diaminobenzidine/hydrogen peroxide reaction, haematoxylin counter stained sections were dehydrated and mounted in DPX.

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Table I—Antibodies used in this study

Antibody	Specificity	Source	Working Dilution
I Mouse monoclonals			
Anti human EMA	Epithelial membrane antigen	Dakopatts	1:40
Anti cytokeratin	Human cytokeratins Moll Catalogue numbers 8, 18, 19	Becton-Dickinson	1:20
Anti CEA	Carcinoembryonic antigen	CIS-UK	Predetermined reconstitution dilution of freeze dried antibody
Anti CA 19-9	Gastrointestinal adenocarcinoma associated tumour marker	CIS-UK	"
Anti Leu M1	-Leu M1 (CD15) a myelomonocytic antigen -Reed-Sternberg cells	Becton-Dickinson	1:40
Polyclonals			
II* Rabbit anti mouse immunoglobulins—peroxidase labelled	Immunoglobulin—Mouse heavy chain and light chains	Dakopatts	1:50
III* Swine anti rabbit immunoglobulins—peroxidase labelled	Rabbit immunoglobulins heavy and light chains	Dakopatts	1:50

*The polyclonal antibody dilutions were made in 5% human AB serum to eliminate any species cross reactivity with human immunoglobulin.

Controls included omission of primary layer monoclonals and replacement of primary antibodies by normal mouse immunoglobulins.

RESULTS

Normal tissue

The reactivity of the anti Leu M1 antibody and other antibodies to epithelial associated antigens with normal tissues are shown in Table II.

Malignant (Neoplastic) tissue

The reactivity of a wide range of neoplastic epithelia with the anti Leu M1 and the other listed monoclonal antibodies are shown in Table III.

Figs 1 and 2 show the reactivity of anti Leu M1 with several epithelial tumours, and comparative results with the other monoclonal antibodies. The

tumours which stained for Leu M1 (CD15) showed varying combinations of apical/and peripheral membrane and cytoplasmic patterns of reaction, similar to those noted for anti-EMA labelling of tumour cells.

DISCUSSION

This study has demonstrated that the monoclonal antibody anti Leu M1, which reacts with epitopes on myelomonocytic cells and on Reed-Sternberg cells, also reacts with determinants expressed by normal and neoplastic epithelia. This immunoreactivity has been compared with other well characterized antibodies to epithelial-associated antigens.

The spectrum of reactivity of anti Leu M1 with epithelium has many similarities to that reported by McCarthy *et al.*⁸ for mouse antigranulocyte monoclonal antibodies designated MC2 and MC4. Some obvious differences are apparent; thus nor-

Table II—Anti Leu M1 staining of normal epithelial tissues compared with antibodies to other epithelial antigens

Tissue	Leu M1	EMA	Cytokeratin	CEA	CA19-19
Epithelium of:					
Stomach	+	+	+	+	+
Colon	+	+	+	+	+
Salivary gland ducts	+	+	+	+	—
Thyroid	—	—	+	+	—
Ecto/endo cervix	—	+	+	+	—
Ovary—surface and follicles	+	+	+	+	±
Endometrium	+	+	+	—	—
Kidney	—	+	+	—	—
Breast	—	+	+	+	—
Fallopian tubes	+	+	+	—	—
Gall bladder	+	+	+	+	+
Skin—epidermis	—	—	—	—	—
Skin—sweat glands	+	+	+	—	+

+ positive staining

— negative staining

Staining was performed on one block of each tissue

Table III—Immunohistochemical reactions of neoplastic epithelium with monoclonal antibody Leu M1 and other epithelial associated antibody markers

Epithelial tumours	Leu M1	EMA	Cytokeratin	CEA	CA19-9
Stomach carcinoma	+	+	+	+	+
Colonic carcinoma	+	+	+	+	+
Pleomorphic salivary adenoma	+	+	+	+	+
Ductal carcinoma breast	+	+	+	+	+
Adrenal carcinoma	+	—	+	—	—
Bladder carcinoma	+	+	+	+	+
Sweat gland tumour	+	+	+	—	+
Mixed lacrimal gland tumour	+	+	+	—	+
Basal cell carcinoma	+	—	—	—	—
Renal clear cell carcinoma	—	—	—	—	—
Prostatic carcinoma	—	+	+	—	+
Follicular carcinoma of thyroid	—	+	+	+	+
Papillary carcinoma of thyroid	—	—	+	+	—
Mucinous carcinoma of breast	—	+	+	+	—
Other tumours					
Pheochromocytoma	+	—	—	—	—

+ positive staining

— negative staining

All Sarcomas were negative for Leu M1 detection

mal salivary duct epithelium are reactive for Leu M1 determinants, but are reported to be negative with MC2 and MC4.

It is known that anti Leu M1 binding to myelomonocytic cells is inhibited by the

oligosaccharide-lacto-*N*-fucopentaose III.⁹ A region of that carbohydrate termed 3 fucosyl-*N*-acetyllactosamine has been shown to be recognized by MC2 and MC4 and by other antigranulocyte antibodies (Dr M. A. Kerr, Dundee—personal

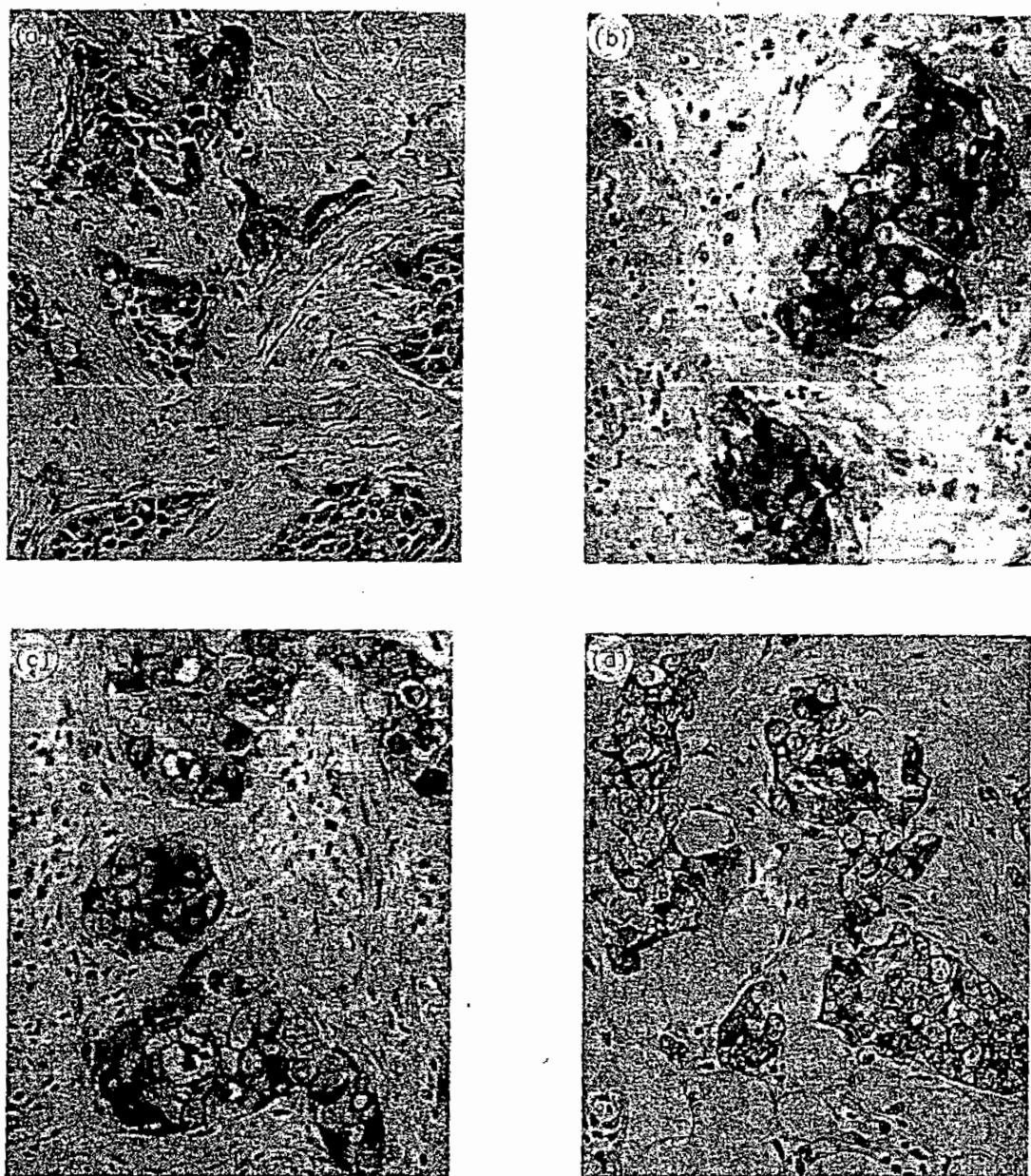


Fig. 1—Breast Carcinoma showing: (a) anti Leu M1 reaction with CD15 determinants; membrane and cytoplasmic staining of the carcinoma cells are evident; (b) anti-EMA gives a more diffuse and intense reaction on the tumour cells; (c) anti-CEA is positive, with less intense and more focal staining; (d) anti-Cytok gave intense cytoplasmic staining

communication). Interestingly, these biochemically characterized determinants have also been demonstrated on adenocarcinoma cell lines.^{10,11} Carbohydrate antigens are known to be stable to many fixation techniques; in retrospect knowledge of the biochemical data, together with the known specificity of anti Leu M1, should have predicted the

likely reaction of this monoclonal antibody with epithelial tumours.

The general finding of the reaction of anti Leu M1 with various carcinomas, adds little information to that derived from the use of the already established markers such as EMA, Cytokeratin and CEA, and therefore anti Leu M1 would not

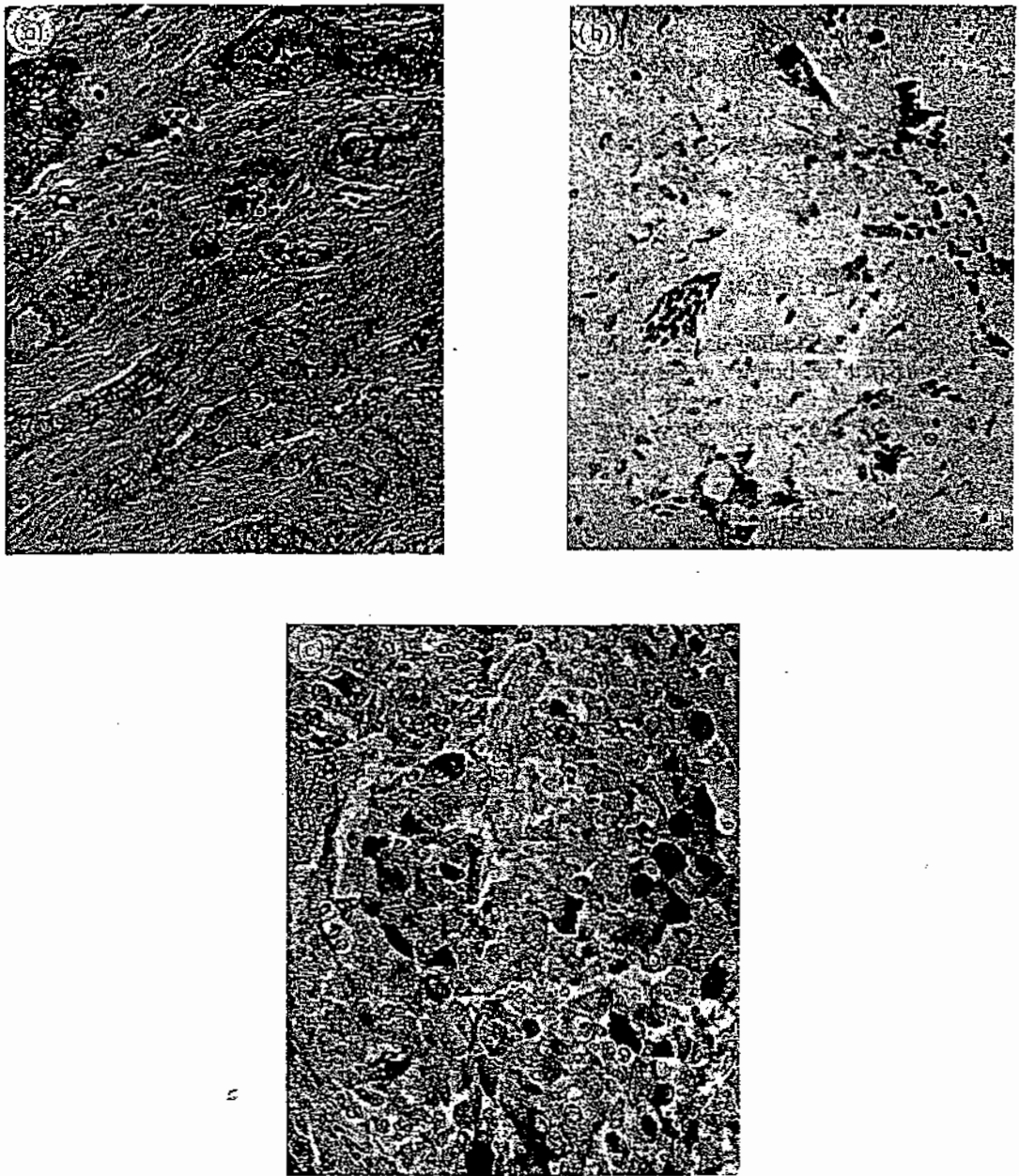


Fig. 2—A variety of tumours stained with anti Leu M1: (a) gastric carcinoma cells stain with anti Leu M1 showing membrane and cytoplasmic staining; (b) pleomorphic salivary adenoma cells show intense staining; (c) pheochromocytoma cells show intense staining

recommended as a routine carcinoma-associated marker. Nevertheless occasions may occur when the reactivity of anti Leu M1 may be exploited. In view of the now documented extensive reactions of anti Leu M1 with myeloid, lymphoid and epithelial cells

of normal and malignant phenotype, it would appear sensible to limit the 'routine' use of this reagent to the investigation of occasional difficult cases of probable Hodgkin's disease. During the course of preparation of this manuscript, two

papers have appeared reporting reactivity of Leu M1 with epithelial tissues.^{12,13}

Finally unexpected reactions of monoclonal antibodies are well documented^{14,15} and even though they may not prove useful in routine diagnosis, as in the present case, in a research context, they may nevertheless generate useful knowledge and possibly indicate new applications.

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