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Monoclonal Antibodies to the Human Mammary Gland

I. Distribution of Determinants in Non-Neoplastic Mammary and Extra Mammary Tissues

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Summary. Mouse monoclonal antibodies have been raised to the human milk fat globule membrane. The distribution of the antigens detected by four of the antibodies has been examined in formalin-fixed, paraffin-embedded human tissues by light microscopic immunocytochemistry. The four antibodies stain lactating breast and normal resting breast. Two exclusively stain the luminal membranes of breast epithelial cells. A third antibody stains in addition the lateral membranes of duct epithelial cells. The fourth antibody stains both epithelial and myoepithelial cells. None of the antibodies is breast specific, nor do they stain every epithelial cell within the breast. Instead, each antibody reveals a complex and heterogeneous distribution of staining throughout the normal tissues. Within the breast, the staining by a given antibody is usually segmental and conforms to secretory units and their associated ducts. Similarly heterogeneous patterns of staining are also observed in the extramammary normal tissues. Despite the apparent morphological identity between breast epithelial cells when examined by conventional light microscopy, the hitherto unrecognised "functional" heterogeneity, which has been revealed by the monoclonal antibodies could have importance in understanding the biology of the normal breast and the pathology of breast cancer.

Key words. Monoclonal antibodies – Milk fat globule membranes – Human breast epithelial cells – Heterogeneity

Introduction

The glands of the mature resting breast contain two basic types of epithelial cells, the luminal epithelial and myoepithelial cells (Bässler 1970). Luminal epithelial cells comprise what appears to be a morphologically homogeneous group

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by conventional light microscopy. However, studies have claimed that several different types of cell can be recognised within this group using electron microscopy (Dabelow 1957; Ozzello 1970, 1971, 1974). Myoepithelial cells are recognised by their topography and ultrastructural characteristics (Haguenau and Arnoult 1959; Hamperl 1970). The presence of transitional cell types or various sub-types of myoepithelial cells remains controversial. Morphological criteria and topographical relationships are clearly inadequate when attempting to relate the organisation and differentiation of the normal breast with structures observed in tumours or in tissue culture.

It was the aim of the present study to produce a range of specific markers which would assist in an attempt to ascertain whether subpopulations of human breast epithelial cells may be recognised, independent of their topographical situation, physiological status or ultrastructural features. These markers were required to locate specific cell-membrane determinants in formalin-fixed, paraffin-embedded tissues in addition to frozen tissue sections and tissue cultures.

The synthesis and secretion of milk by breast epithelial cells represents their most functionally differentiated state (Vorherr 1974). During this process, membranes are sequestered from the cytoplasmic component of milk secretion, in addition to luminal plasma membranes (Bargmann and Knoop 1959). Using human milk fat globule membrane as the immunogen, we have made 19 mouse monoclonal antibodies that bind to the luminal epithelial cells of the breast. The antibodies were selected by their binding to a sample of the original membrane preparations and the specificities of their staining tissue sections of human breast. We report the immunocytochemical staining of normal tissues by four of these monoclonal antibodies. The distribution of staining indicates that the four antibodies are directed to four different determinants.

Materials and Methods

i) Milk Fat Globule Membranes

Human milk-fat globule membranes (MFGM) were prepared from fresh, pooled human milk according to Chien and Richardson (1967) and Plantz (1973). A single preparation was used for both immunisation and screening. Protein content of the MFGM was determined according to Lowry (1951).

ii) Monoclonal Antibodies

BALB/c mice were immunised by two intraperitoneal injections of 100 μg MFGM (Protein concentration) in 200 μl Freund's complete adjuvant. The second immunisation was performed seven days after the first. Spleen cells were prepared four days after the second injection and fused with the mouse myeloma NS1 using poly(ethylene-glycol) according to the method of Galfre et al. (1977) as modified by Edwards (1980). Hybrids were grown from the outset as clones in agar in 48 × 2 ml wells, with liquid medium overlay.

Visible hybrid colonies were picked from the agar and transferred individually to fresh wells in liquid culture medium. Culture supernatants from the picked colonies were screened against MFGM one week later, using the MFGM plate binding assay. 42 positive wells were identified. After preliminary characterisation of the specificity of the antibodies, hybrids were recloned by limiting dilution. Those chosen for detailed study by preliminary immunocytochemical staining using concentrated culture supernatants were grown as ascitic tumours in mice.

iii) Nomenclature

Monoclonal antibodies are designated with the prefix LICR LON/M, meaning *L*udwig *I*nstitue for *C*ancer *R*esearch, *LON*don Branch. M refers to *M*ilk fat globule membrane. The number indicates the specific hybrid within the 42 positive hybrids identified by the second plate-binding assay. Hybrids 3, 8, 18 and 24 are discussed in this paper.

The class and sub-class of each antibody was determined by the double diffusion method of Ouchterlony using a 1% agar gel. Sub-class-specific antisera were obtained from Miles Laboratories. Monoclonal antibody LICR LON/M8 is an IgG. Monoclonal antibodies LICR LON/M3, M18 and M24 are of class IgM.

iv) MFGM Plate Binding Assay

Culture supernatants were assayed for specific antibody using MFGM adsorbed onto the surface of plastic tissue culture plates. The plates (24-well 'multiwell', Costar), were prepared by treating the wells with a solution of poly-L-lysine in 100 µl distilled water and incubated overnight at 4° C in a moist chamber. The plates were washed three times with phosphate buffered saline (pH 7.4) before addition of the MFGM. The wells were then coated with a suspension of MFGM diluted in phosphate buffered saline (pH 7.4) and containing 1 mM Phenylmethylsulfonylfluoride (Sigma, Poole, Dorset, GB). 100 µl of this suspension, containing 100 µg protein, was added to each well. The plates were incubated for 24 h at 4° C in a moist chamber. The coated plates prepared in this manner could be stored at 4° C for up to 14 days without detectable deterioration. Prior to use, the plates were again washed three times with phosphate buffered saline in order to remove non-adsorbed membranes. After the final washing, the plates were inverted to allow excess buffer to drain. To each well was then added 50 µl isotonic phosphate buffer (pH 7.4) and 20 µl tissue culture supernatant. The plates were gently agitated at room temperature for one hour. After washing three times with phosphate buffered saline (pH 7.4) containing 1% bovine serum albumin (Fraction V, Miles Laboratories Slough Bucks, GB) the plates were allowed to drain. 200,000 c.p.m. of ^{125}I-labelled rabbit anti-(mouse immunoglobulin G) (Jensenius and Williams 1974) diluted in phosphate buffered saline containing 1% bovine serum albumin was added to each well and incubated for one hour. Following incubation, The plates were washed with three changes of phosphate buffered saline containing 1% bovine serum albumin. One millilitre 0.1% Sarkosyl in 0.1 N NaOH was added to each well and incubated for 10 minutes and the solubilized labelled antibody was taken for gamma-counting. Positive wells were identified as those giving in excess of 15,000 c.p.m. bound. Blanks, negative wells and some weakly positive wells gave less than 250 c.p.m. bound.

v) Immunocytochemical Methods

Human tissues were obtained from three sources. Representative blocks of fresh breast tissue were taken from surgical reduction mammaplasties. Uninvolved breast tissue adjacent to tumours was not used. Fresh normal, extra-mammary tissues were obtained following the surgical removal of organs for non-malignant conditions, or following trauma. The spectrum of human tissues was completed with material taken from fresh autopsies. Surgically-derived tissue was, wherever possible, used in preference to the autopsy material.

Tissue sections were routinely stained with ascitic fluid from recloned hybrids diluted 1:100 with phosphate buffered saline. 5 µ sections of formalin-fixed (10% buffered formol saline) paraffin-embedded tissues were de-waxed with xylene and taken to water through ethanol. Endogenous alkaline phosphatase activity was blocked by exposure to 15% glacial acetic acid in distilled water for 15 min. The sections were then rinsed in phosphate buffered saline (pH 7.4) containing 0.5% Tween 80 and all subsequent washings were performed with this solution. Excess buffer was wiped from around the sections and the slides placed horizontally in a moist chamber. 100 µl aliquots of the concentrated culture supernatants were carefully pipetted onto the sections so that the surface of the tissues were covered uniformly and the sections were incubated at room temperature for 1 h. The sections were washed three times, drained and excess buffer wiped from around the tissue. 100 µl sheep anti-(mouse immunoglobulin G) conjugated to alkaline phosphatase (Type VII, Sigma) (Avrameas and Ternynck 1971) and diluted in phosphate buffered saline containing 1% bovine serum albumin was then pipetted onto the sections and incubation continued 1 h before the sections were rinsed. The slides were finally liberally covered with a freshly-prepared mixture

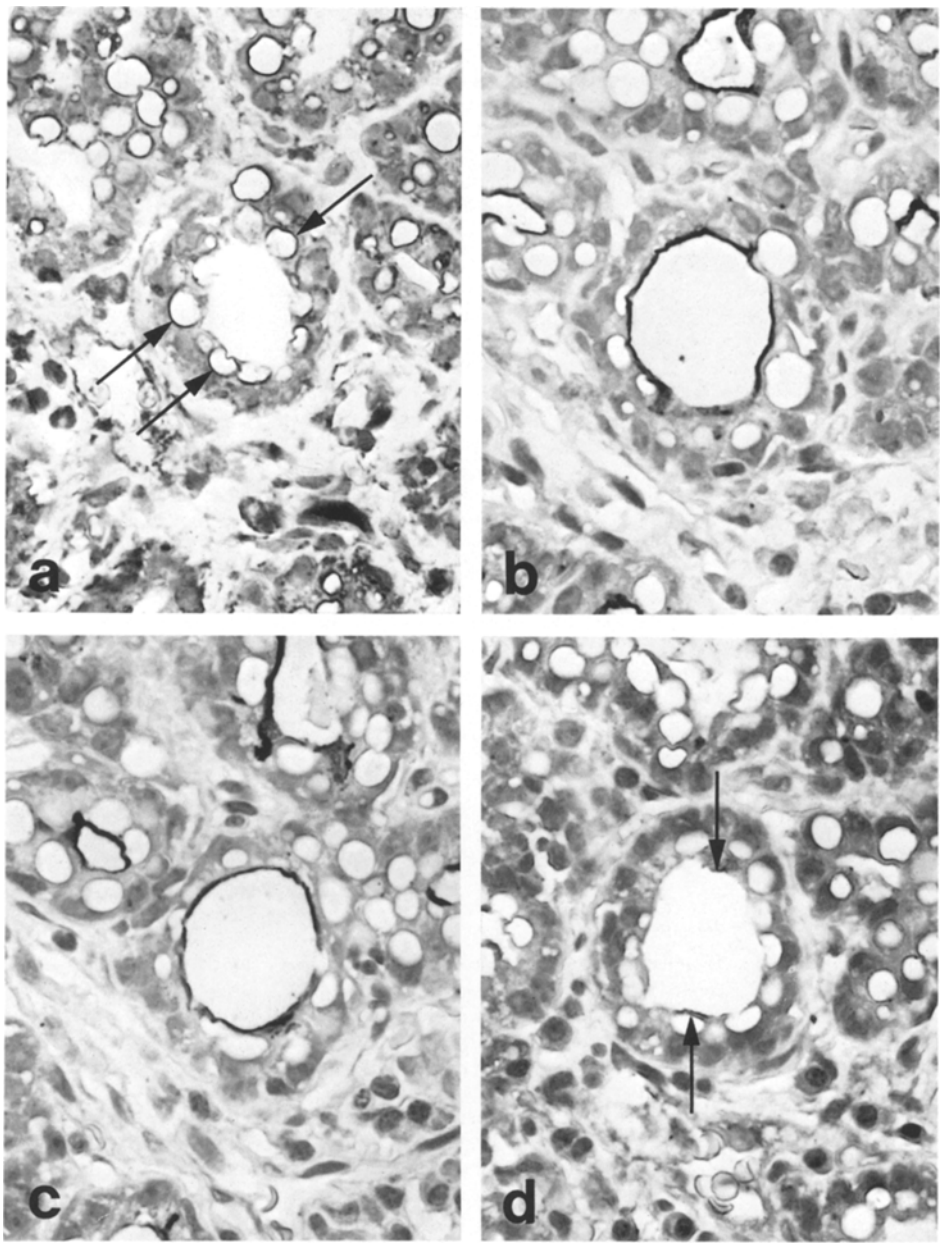

Fig. 1a–d. Adjacent sections of lactating breast stained by monoclonal antibodies to the MFGM. (**a**) LICR LON/M3: The antibody is distributed around the membrane of all intracytoplasmic milk fat globules (*arrows*) and not the luminal membranes of the epithelial cells. (**b**) LICR LON/M8, (**c**) LICR LON/M18: These antibodies localise the luminal membranes of the lobular epithelial cells. The milk fat globules are not stained. (**d**) LICR LON/M24: This antibody is heterogeneous in staining the lactating breast. In this section only two cells stained (*arrows*). (Alkaline phosphatase with Meyer's haemalum counterstain. ×800)

Table 1. LICR LON/MFGM monoclonal antibodies. Specificities of staining breast tissues

Tissue	Site	Antibody			
		M3	M8	M18	M24
Normal adult female resting breast	*Acini*				
	Luminal membranes	+	+	+	+
	Interepithelial cell membranes	+	−	−	−
	Myoepithelial cells	+	−	−	−
	Intralobular ducts				
	Luminal membranes	+	+	+	+
	Interepithelial cell membranes	+	−	−	+
	Myoepithelial cells	+	−	−	−
	Interlobular ducts				
	Luminal membranes	+	+	+	+
	Interepithelial cell membranes	+	−	−	+
	Myoepithelial cells	+	−	−	−
	Lactiferous sinuses				
	Luminal membranes	+	+	+	+
	Interepithelial cell membranes	+	−	−	−
Lactating breast	*Acini*				
	Luminal membranes	−	+	+	+
	Milk globule membranes	+	−	−	−
	Interepithelial cell membranes	−	−	−	−
Male gynecomastia	*Acini*				
	Luminal membranes	+	+	−	−
	Interepithelial cell membranes	+	−	−	−
	Myoepithelial cells	+	−	−	−
Breast vascular tissue	Endothelium	−	−	−	−
	Pericytes	+	−	−	−

of Fast Red (Sigma) (0.5 mg/ml) and sodium naphthol As-Bl (Sigma) (0.5 mg/ml) in veronal acetate buffer pH 9.2 and incubated for 45 min. After thorough washing, the sections were counterstained in buffered Meyer's haemalum, blued in saturated lithium carbonate and mounted in warm glycerin jelly.

The staining of the histological sections was controlled in two ways. Possible non-specificity of monoclonal antibody staining was performed using serial sections of three samples of normal breast tissue, each from a different subject. Furthermore, the observed staining of the serial sections by each of the antibodies conformed to a uniform pattern. The presence of endogenous alkaline phosphatase, non-specificity of the second-antibody-enzyme-conjugate and possible non-specific adherence to the sections of the visualisation reagents was excluded using these solutions individually. Non-specific staining by these reagents was not observed.

Results

i) Breast Tissues

All four monoclonal antibodies selected for this study stained paraffin sections of lactating breast (Fig. 1) and normal breast tissues. A detailed analysis of

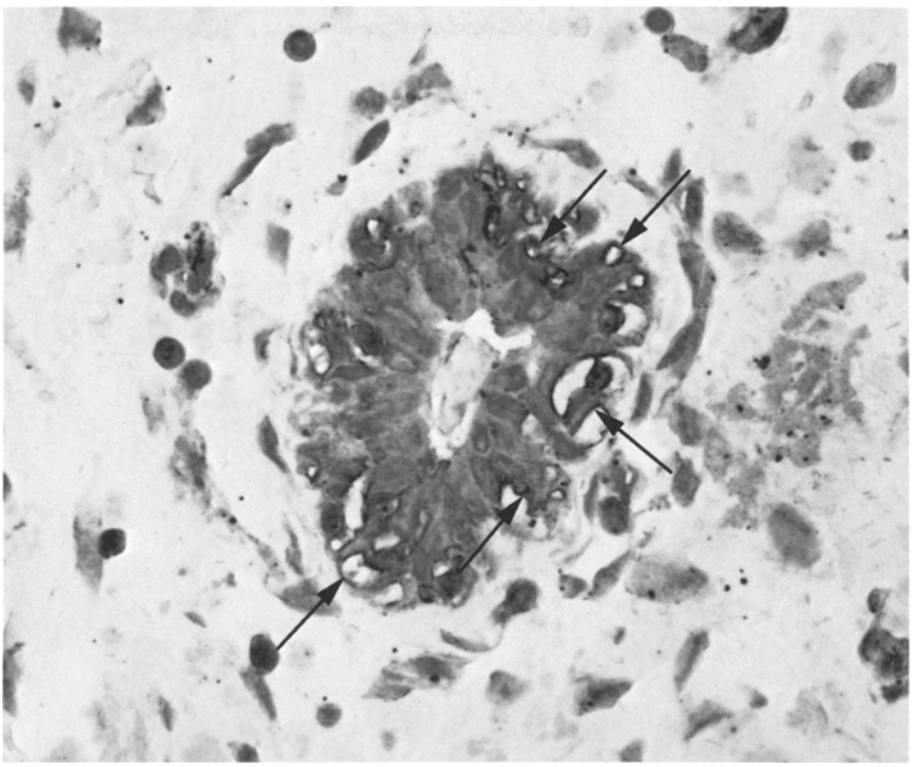

Fig. 2. Normal breast tissue stained by monoclonal antibody LICR LON/M3. The myoepithelial cells of the small duct were stained heavily with this antibody (*arrows*). The luminal epithelial cells of this duct showed no staining. ($\times$ 1,000)

the staining patterns is presented in Table 1. One of the antibodies (LICR LON/M3) weakly stained myoepithelial cells in addition to the luminal epithelial cells of both types of breast tissue (Fig. 2). This antibody also stained the membrane of the intracytoplasmic milk fat globule of lactating breast (Fig. 1a). The other three monoclonal antibodies did not stain myoepithelia (Fig. 3). One variation in staining was observed with monoclonal LICR LON/M24. In the lobules of the lactating and normal breast, the antigen detected by this antibody was restricted to the luminal membranes. However, in the ducts the antigen was also detected along the interepithelial (lateral) cell membranes (Fig. 4). Within the breast, staining by monoclonal antibody LICR LON/M3 was not restricted to the epithelium, but also included pericytes surrounding blood vessels. Vascular endothelium did not stain.

The staining of both lactating breast and normal breast epithelial cells was heterogeneous with each monoclonal antibody. None of the antibodies stained every luminal epithelial cell within the tissue sections. Particularly in the sections of resting breast, staining varied between adjacent cells, as well as between adjacent regions of the same breast. Thus, individual cells could be stained intensely with a given antibody while adjacent cells remained unstained. The

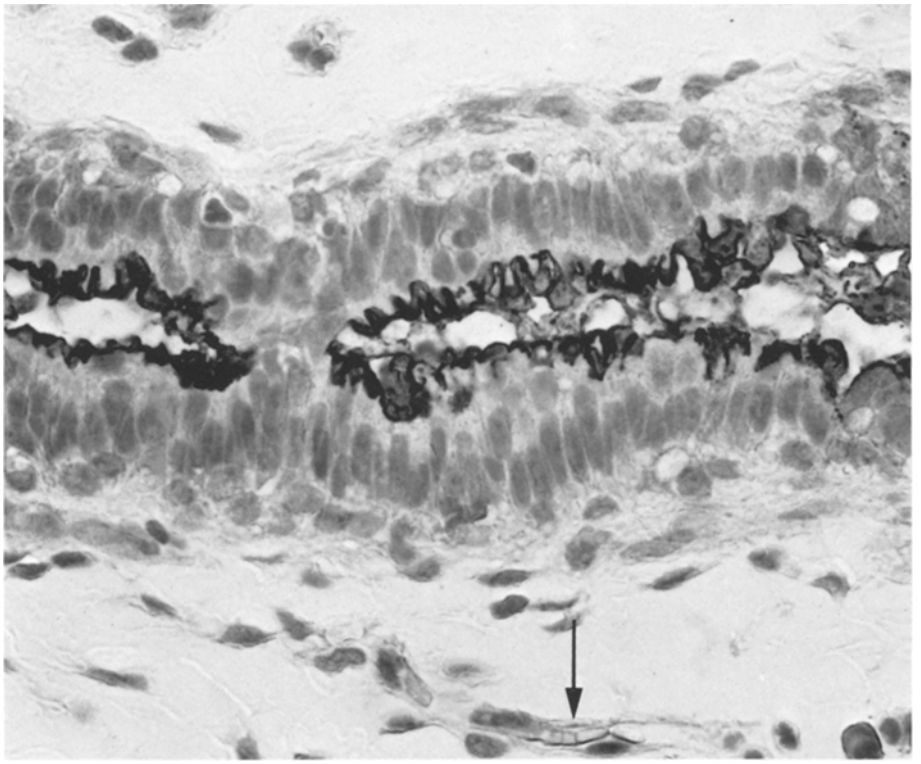

Fig. 3. Longitudinal section of a normal breast duct stained by monoclonal antibody LICR LON/M8. The staining is resctricted to the luminal surface of the epithelial cells, and does not penetrate between the epithelial cells. The myoepithelial cells are not stained. The small capillary and the contained erythrocytes are not stained (*arrows*). (×800)

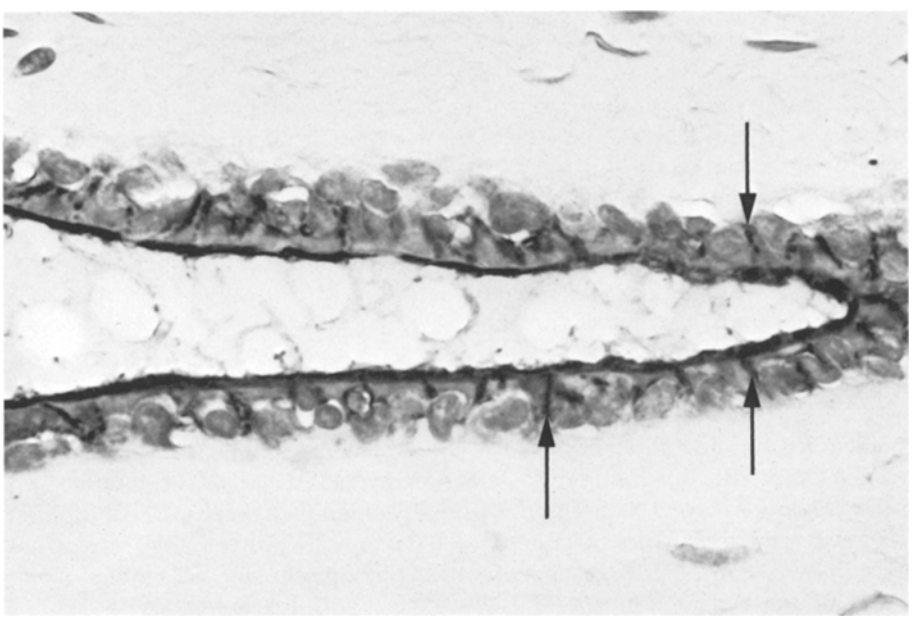

Fig. 4. Longitudinal section of a normal breast duct stained by monoclonal antibody LICR LON/M24. In addition to the luminal membranes, the lateral membranes of the epithelial cells are also stained (*arrows*). Basal membranes and myoepithelial cells are not stained. (×760)

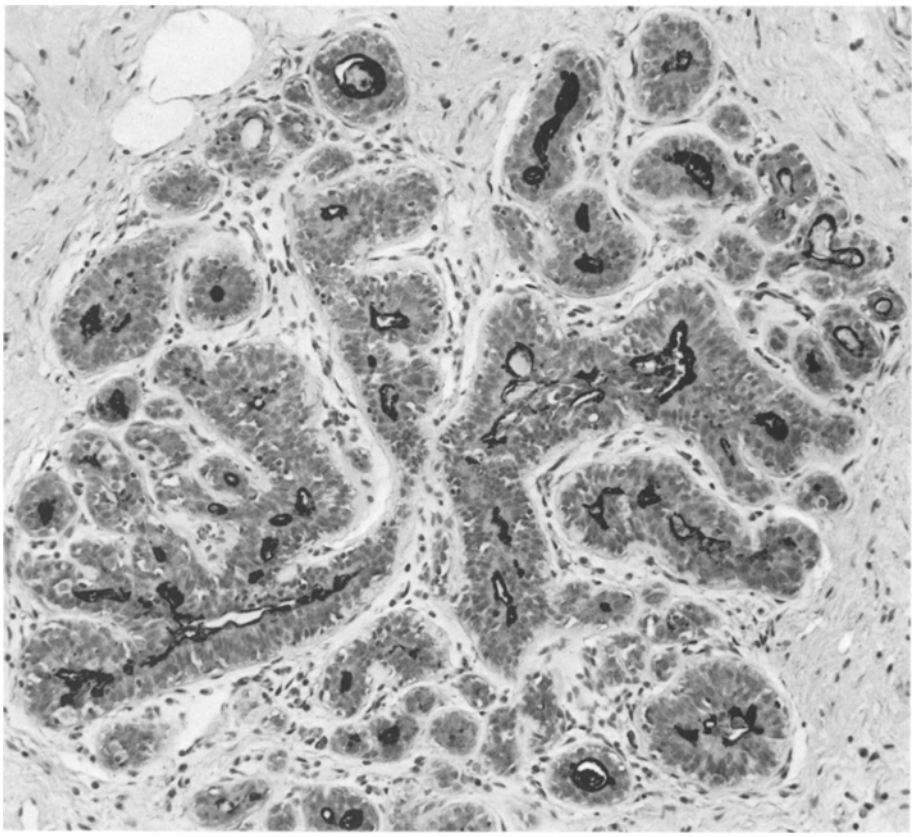

Fig. 5. A single secretory unit within a section of normal breast stained by monoclonal antibody LICR LON/M8. The luminal membranes of the majority of the epithelial cells are stained. (×120)

sequential staining of serial sections with different monoclonal antibodies demonstrated that, in general, the staining always occurred in the corresponding regions of adjacent sections. Usually, these regions could be defined as anatomical units within the breast. For example, in a particular part of a section the staining might be restricted to an interlobular duct, and the attached secretory units (Fig. 5). Alternatively, the lobules of one secretory unit might be stained, with infrequent staining of the epithelial cells lining the intralobular and interlobular ducts supplying that unit.

ii) Extramammary Tissues

The detailed staining patterns of the four monoclonal antibodies are presented in Tables 2–5. All four antibodies stained epithelia. Although the staining was restricted to the plasma membranes of the breast epithelial cells, the cytoplasm of some extramammary epithelia were stained intensely. For example, monoclonal antibody LICR LON/M18 stained both the cytoplasm and the luminal membranes of the zymogenic cells of the stomach and LICR LON/M24 stained

Table 2. LICR LON/MFGM monoclonal antibodies. Specificities of staining in the integument

	M3	M8	M18	M24
Keratin	[a]	−	−	−
Stratum granulosum	+	−	+	−
Stratum spinosum	+	−	−	−
Stratum germinativum	[a]	−	−	−
Sweat glands	+	+	−	+
Sweat ducts	+	+	−	+
Sebaceous glands	+	+	+	−

[a] Very weak staining

Table 3. LICR LON/MFGM monoclonal antibodies. Specificities of staining gastrointestinal tract

Organ	Cell type	Antibody			
		M3	M8	M18	M24
Oesophagus	Surface epithelium	+	−	−	[a]
Stomach	Secretory epithelium	−	−	−	−
	Non-secretory epithelium	+	+	+	+
	Parietal cells	+	+	−	+
	Zymogenic cells	−	+	+	−
Ileum	Surface epithelium	+	−	−	+
	Crypt epithelium	+	−	−	+
Colon	Surface epithelium	+	+	+	+
	Crypt epithelium	+	−	−	−
Liver	Parenchyma	−	+	−	−
	Kupffer cells	−	−	+	−
	Bile ducts	+	+	−	+
Pancreas	Exocrine acinar cells	−	+	−	+
	Centro acinar cells	+	+	−	+
	Exocrine duct lamina	+	−	−	+
	Islet cells	+	−	−	−

[a] Very weak staining

intensely the cytoplasm of the centroacinar cells of the pancreas. Antibody LICR LON/M3 also stained tissues derived from the embryonic mesenchyme, particularly smooth muscle, skeletal muscle and adrenal cortex. In addition to epithelia, antibody LICR LON/M18 also stained the adrenal cortex and the small penetrating capillaries of the brain. The large intracerebral vessels, and the extracerebral vessels did not stain. The staining of normal tissues by antibodies LICR LON/M8 and LICR LON/M24 was exclusively epithelial and included both ectodermal and endodermal derivatives.

The distribution of staining the range of normal tissues was unique for each antibody. None of the antibodies stained every cell within a particular epithelium. Not only were the variations in staining cell-type specific (e.g. zymogenic vs. parietal cells in the stomach, Table 3), but they were often focal

Table 4. LICR LON/MFGM monoclonal antibodies. Specificities of staining genitourinary tissues

Organ	Cell type	Antibody			
		M3	M8	M18	M24
Uterus	Gland epithelium	+	+	+	−
	Stroma	+	−	−	−
Fallopian tube	Epithelium	+	+	+	+
	Stroma	+	−	−	−
Cervix	Endocervix epithelium	+	+	+	−
	Ectocervix epithelium	+	−	−	−
	Stroma	+	−	−	−
Ovary	Follicles	−	−	−	−
	Stroma	+	−	−	−
Prostate	Glands	−	−	−	−
	Ducts	+	+	−	−
	Stroma	+	−	−	−
Testis	Spermatogonia	+	+	−	+
	Spermatocytes	+	+	+	+
	Spermatozoa	−	+	+	+
	Leydig cells	+	+	−	+
Kidney	Glomeruli	−	−	−	−
	Proximal tubules	[a]	[a]	[a]	[a]
	Distal tubules	[a]	+	+	[a]
	Collecting ducts	+	+	+	+

[a] Very weak staining

Table 5. LICR LON/MFGM monoclonal antibodies. Specificities of staining respiratory, nervous and endocrine tissues

Organ	Cell type	Antibody			
		M3	M8	M18	M24
Trachea	Respiratory epithelium	+	+	+	+
	Glands	+	+	+	+
Lung	Alveolar epithelium	−	+	+	−
Brain	Neurones	+	[a]	−	−
	Glia	−	−	−	−
	Capillaries	−	−	+	−
	Peripheral nerves	−	−	−	−
Adrenal	Cortex	+	−	+	−
	Medulla	+	−	−	−
Thyroid	Epithelium	+	+	+	−
	Colloid	−	−	−	−

[a] Very weak staining

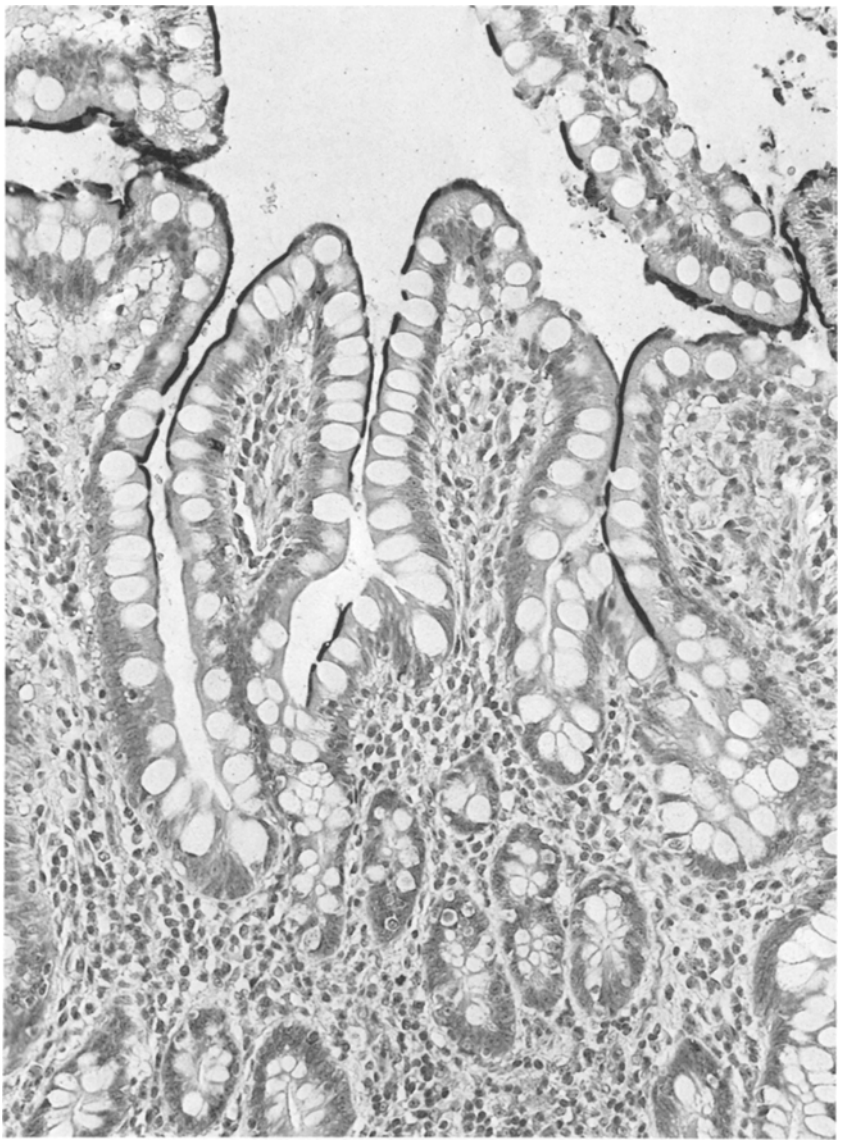

Fig. 6. Section of small intestinal mucosa stained by monoclonal antibody LICR LON/M8. The luminal membranes of the non-secretory surface epithelium are stained intensely. Cells in the deep regions of the crypts are not stained. (×160)

within a particular epithelium. For example, the staining of the parietal and zymogenic cells of the stomach by LICR LON/M8 was focal, and recurred at intervals along the epithelium. Within every epithelium which stained, each antibody produced a heterogeneous pattern which was unique for that antibody. The heterogeneity observed within extramammary epithelia thus resembled that

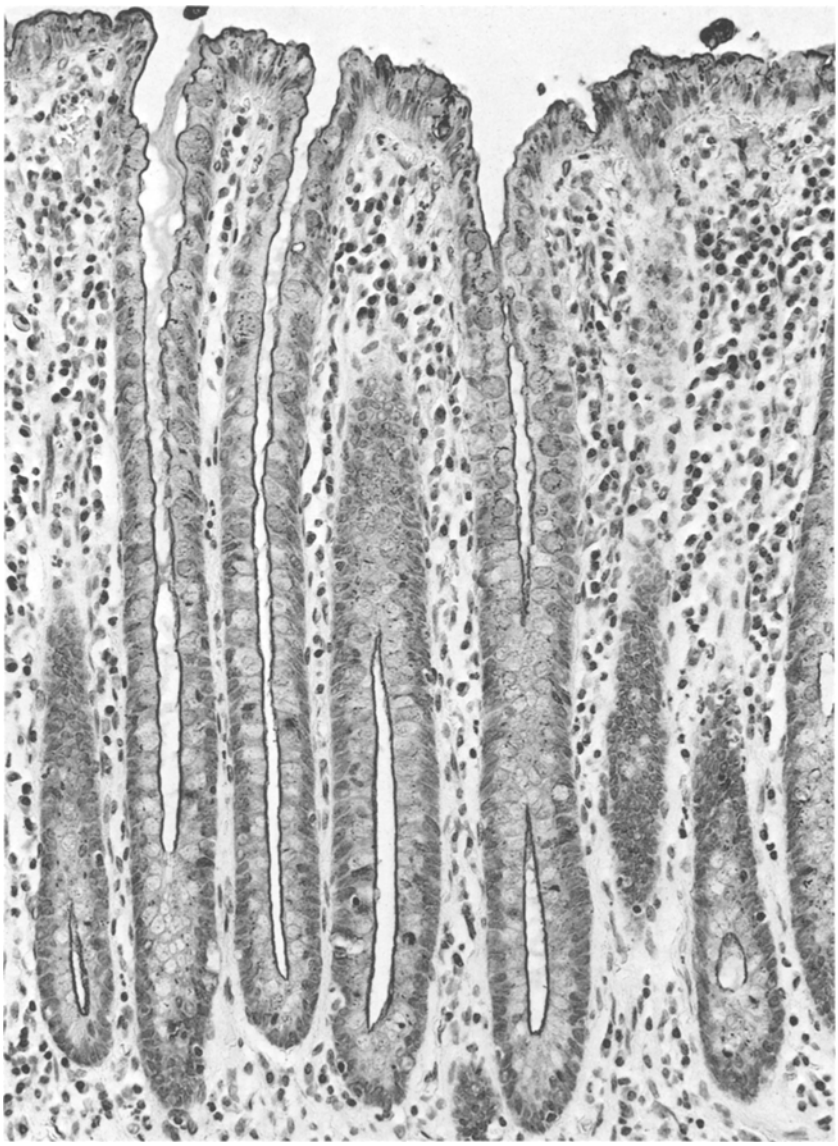

Fig. 7. Section of rectal mucosa stained by monoclonal antibody LICR LON/M3. The luminal membrane of all surface epithelial cells stain with this antibody, irrespective of their position in the crypts. (× 140)

already described for the breast epithelium. In addition to the focal variations, these monoclonal antibodies frequently demonstrated changes in staining which could be directly correlated with cell maturation or topography. Monoclonal antibody LICR LON/M8 stained only the luminal plasma membranes of the nonsecreting cells in the surface layer of the ileal and colonic mucosa (Fig. 6). Morphologically identical cells within the crypts did not stain. A similar pattern

was produced in the colon by antibody LICR LON/M24. The staining of the endometrial glands by antibody LICR LON/M3 was the reverse pattern. The staining was most intense in the deep portion of the glands, but was absent from the surface of the endometrium. In the colon, this antibody stained the luminal plasma membranes of all the mucosal epithelial cells, irrespective of their position in the crypts (Fig. 7).

Some tissues were stained uniformly by monoclonal antibodies. These tissues were invariably derived from embryonic mesenchyme. For example, all smooth and skeletal muscle cells appeared homogeneous in staining by LICR LON/M3, and LICR LON/M18 uniformly stained all the cells of the adrenal cortex.

Discussion

A detailed examination of the distribution of antigens identified by the four monoclonal antibodies to the MFGM has revealed the determinants to be present on some extramammary tissues, in addition to the epithelial cells of normal and lactating breast. Two of the antibodies have also identified determinants in tissues derived from mesenchyme as well as in epithelia. Within the epithelia of the breast and other normal tissues the staining patterns of the monoclonal antibodies has revealed hitherto unrecognised heterogeneity. Not only are gross regional variations identified by the antibodies, but also differences between morphologically identical adjacent cells within a single region. The demonstration, by means of serial sections, that the staining patterns define anatomical regions and remain constant is an indication that this is a real biological phenomenon, and not an artifact of fixation or staining.

One problem which is well recognised by histochemists is the variability of staining produced by incomplete fixation of tissues prior to embedding (Wallington 1979). The effect of fixation was determined using frozen sections of fresh normal breast tissue stained by these four monoclonal antibodies. The sections were unfixed or fixed in buffered formol saline prior to staining. The observed patterns of staining were similar to those obtained following the use of formalin fixed, paraffin-embedded tissues. In this study the tissues were fresh, trimmed into small pieces and fixed adequately in a standard manner before further processing. Care was always taken to ensure that the tissue sections were completely covered by the reagents employed during the staining and that at no time were the reagents allowed to evaporate. The presence of intense staining located to anatomical regions (for example secretory units and the associated ducts) and pronounced differences between adjacent cells strongly suggests that the resultant patterns indicate important variations in the surface membrane composition at that time. The similarly heterogeneous patterns of staining observed throughout the other normal epithelia indicate that variability in the expression of surface determinants is a feature common to all epithelial cells, not only breast.

Milk fat globule membranes prepared in aqueous, inorganic media comprise a heterogeneous combination of carbohydrate, protein and lipid antigens. A specific determinant identified by a monoclonal antibody (defined as an epitope) may be present as part of more than one larger molecule within the MFGM.

For example, a particular carbohydrate epitope may form a part of a glycoprotein and a glycolipid, simultaneously. The two molecules may have different functions and be distributed in different sites within a cell. However, within the breast it is possible to make statements concerning the expression and distribution of determinants contained within the milk fat globule membrane. No information is presently available concerning the structure of the determinants within the MFGM, or whether those determinants form part of the same antigens in the extramammary tissues. Nevertheless, the differential distribution observed in the staining of normal tissues by the four monoclonal antibodies demonstrates that each antibody is directed towards a different epitope. The various distributions cannot be explained by two or more of the antibodies having the same epitope specificity, but different affinities. It is possible, however, that within the MFGM, at least two of the antibodies may identify two separate epitopes of the same molecule, but that these epitopes are expressed in a different association in the extramammary tissues. These and other monoclonal antibodies may be used as probes with which to investigate the biology of breast and other human tissues. Provided the experimental systems are well defined, and the appropriate antibody is chosen, it is of little or no consequence that the determinants identified by the antibodies are also expressed by other tissues. Thus, these reagents may assist in relating observations on tumours and tissue culture to the normal tissue.

The synthesis and secretion of milk by breast epithelium, probably represents the most functionally differentiated state of the individual epithelial cells (Vorherr 1974). Therefore, antibodies which identify components of membranes produced during lactation define a very specific state of functional differentiation of the breast cells expressing these determinants. Hence, the four monoclonal antibodies which have been raised to the milk fat globule membrane demonstrate a functional heterogeneity within both the lactating breast and the normal breast which was hitherto unrecognised. The staining patterns produced by the four antibodies indicate that cells which are morphologically and topographically identical may simultaneously coexist in at least two different states of functional differentiation. Moreover, the heterogeneity which has been defined by these monoclonal antibodies suggests that between those populations of epithelial cells, other antigenic distinctions may also occur. The expression of particular cell-surface determinants by morphologically identical cells may represent fluctuations in the functional status of those cells with time. Alternatively, the expression of the determinants may define specific and permanent subpopulations within those epithelial cells.

We intend to use these antibodies to investigate functional differentiation of cells within breast tumours and breast tissue culture in an attempt to relate these observations to the normal breast. The possible significance of the heterogeneity to breast cancer is discussed in the accompanying paper (Foster et al. 1982).

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References

Avrameas S, Ternynck T (1971) Peroxidase labelled antibody and Fab conjugates with enhanced intracellular penetration. Immunochemistry 8:1175–1179

Bargmann W, Knoop A (1959) Über die Morphologie der Milchsekretion. Licht- und elektronenmikroskopische Studien an der Milchdrüse der Ratte. Z Zellforsch 49:344–388

Bässler R (1970) The morphology of hormone induced structural changes in the female breast. Curr Top Pathol 53:1–89

Chien HC, Richardson T (1967) Gross structure of the globule membrane of cow's milk. J Dairy Sci 50:451–455

Dabelow A (1957) Die Milchdrüse. In: Bargmann W (Hrsg) Handbuch der mikroskopischen Anatomie des Menschen. Vol 3, part 3. Haut und Sinnesorgane. Springer, Berlin, Göttingen, Heidelberg. S 277–485

Edwards PAW (1980) Monoclonal antibodies that bind to the human erythrocyte-membrane glycoproteins glycophorin A and Band 3. Proc Biochem Soc Trans 8:334–335

Foster CS, Dinsdale E, Edwards PAW, Neville AM (1982) Monoclonal antibodies to the human mammary gland: (II) Distribution of determinants in breast carcinomas. Virchows Arch [Pathol Anat] 394:295–305

Galfre G, Howe SC, Milstein C, Butcher GW, Howard JC (1977) Antibodies to major histocompatibility antigens produced by hybrid cell lines. Nature (Lond) 266:559–552

Haguenau F, Arnoult J (1959) Le Cancer du sein chez la femme. Bull Ass Franç Cancer 46:177–211

Hamperl H (1970) The myothelial (myoepithelial cells). Normal state; regressive changes; hyperplasia; tumours. Curr Top Pathol 53:162–220

Jensenius JC, Williams AF (1974) The binding of anti-immunoglobulin antibodies to rat thymocytes and thoracic duct lymphocytes. Eur J Immunol 4:91–97

Lowry OH, Rosebrough NJ, Lewis-Farr A, Randall RJ (1951) Protein measurement with the Folin-Phenol reagent. J Biol Chem 193:265–275

Ozzello L (1970) Epithelial-stromal junction of normal and dysplastic mammary glands. Cancer 25:586–600

Ozzello L (1971) Ultrastructure of the human mammary gland. In: Sommers SC (ed) Pathology annual 1971. Appleton-Century-Crofts, New York, pp 1–59

Ozzello L (1974) Electron microscopic study of functional and dysfunctional human mammary glands. J Invest Dermatol 63:19–26

Plantz PE, Patton S, Keenan TW (1973) Further evidence of plasma membrane material in skim milk. J Dairy Sci 56:978–983

Vorherr H (1974) The Breast: morphology, physiology and lactation. Academic Press, New York

Wallington EA (1979) Artifacts in tissue sections. Med Lab Sci 36:3–61

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