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Expression of major histocompatibility antigens and leucocyte infiltration in benign and malignant human breast disease

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Summary The reactivity of murine monoclonal antibodies (McAbs) directed against the monomorphic determinants of Class I and Class II antigens of the major histocompatibility complex (MHC), and against antigens expressed by discrete populations of leucocytes was studied using the indirect immunoperoxidase technique on serial tissue sections of 16 benign and 17 malignant primary human breast tumours. Class I antigens (detected by the McAb 2A1) were consistently associated with stromal leucocytes, fibroblasts and vascular endothelium, but expression on epithelial cells particularly of malignant provenance, was more variable. Class II antigens (detected by TDR 31.1) were present upon a variety of cell types which also included sporadic expression on malignant and benign epithelia. The distribution of leucocytes grossly monitored with 2D1 (reactive with a common leucocyte antigen) was largely interepithelial and periductal in benign lesions. Leucocytes were generally more numerous in malignant tumours, where they were largely confined to the stroma. The majority (~75%) of leucocytes were T lymphocytes (reactive with UCHT1), some of which appeared to react with TDR 31.1 and were therefore activated. Ratios of helper/inducer ($OKT4^+$) and suppressor/cytotoxic ($OKT8^+$) subsets generally exceeded unity in malignant neoplasms. There was no correlation between the extent and distribution of T cells and the HLA status of the epithelial cells. Leucocytes detected by the monoclonal antibody OKM1 which reacts with monocytes/macrophages, granulocytes and large granular lymphocytes were numerically few and again mainly confined to the stroma. In a limited number of tests, leucocytes detected with HNK1, reactive with a differentiation antigen expressed on some cells which mediate natural and antibody-dependent cellular cytotoxicity *in vitro* although detectable interepithelially in benign tumours, were virtually absent from malignant tissue. HNK1 also cross-reacted with myoepithelial cells in the ducts of benign lesions.

Human malignant neoplasms not infrequently reveal mononuclear cell infiltrates which have features in common with immune-associated inflammatory reactions (Underwood, 1974). Although there is limited evidence for tumour-related functional activity in the infiltrating leucocyte compartment (see Haskill, 1983), the relationship of the phenomenon at a functional or histological level to biological behaviour remains largely obscure.

The majority of solid human neoplasms are characterised by marked cellular heterogeneity and any provisional assignment of *in situ* function will require the elucidation of the heterogeneity and microanatomical distribution of potential leucocytic effector cells relative to the tumour population. Such an analysis must also extend to the neoplastic compartment itself. Human tumour cells are phenotypically diverse (Woodruff, 1983) and although several useful markers exist (Lennox & Sikora, 1982) the antigens which evoke cellular immune responses have not been serologically defined.

The initiation of the present study coincided with reports of heterogeneity of expression of Class I (Fleming *et al.*, 1981) and anomalous expression of Class II (DR) antigens (Natali *et al.*, 1981*a*; Daar *et al.*, 1982) of the major histocompatibility complex (MHC) on primary human tumours. Since these MHC products have a crucial role in cell:cell interactions involving both the inductive and effector phases of the immune response it seemed reasonable to examine, as a preliminary approach, the HLA status of primary tumours in conjunction with leucocyte infiltration. For this purpose we have deployed the indirect immunoperoxidase technique using monoclonal antibodies (McAbs) against the major leucocyte populations and their subsets and McAbs to the monomorphic determinants of the HLA Class I and Class II antigens. Breast was the tissue of choice on account of the availability of benign and malignant tissue from the same organ and the absence of infection.

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We report here that the T lymphocyte-predominant leucocyte infiltrates of malignant tumours are quantitatively greater than those of benign neoplasms, there is no simple correlation with the expression of either MHC Class I or Class II antigens on the tumour cells; that the lymphocytic reaction, comprising T-helper and T-suppressor cells, usually in order of their frequency in peripheral blood, is confined mainly to the stroma and that the numbers of *potential* effector cells of whatever provenance (lymphoid or myelomonocytic) within the tumour mass are small.

Patients and methods

Patients

The patients were all admitted for excision/frozen section of a breast lump. Diagnosis was made on the frozen section and in all but one of the malignant cases mastectomy with or without axillary lymph clearance was carried out. Histological diagnosis was confirmed on paraffin sections.

Of the patients with benign breast lesions the age range was 22–65 years. Two patients (050 and 048) were taking oral contraceptives at the time of operation; none of the others were taking steroidal or anti-inflammatory drugs. In the malignant group the age range was 50–78 years.

One patient (046) had had a previous left mastectomy for carcinoma of the breast 6 years before with radiotherapy (left side only) and stilboestrol therapy. At the time of the right mastectomy (for a histologically similar tumour) the patient was taking tamoxifen only.

One patient (049) had a history of radiotherapy to the genito-urinary tract 15 years previously for an unknown reason.

One patient (022) with longstanding rheumatoid arthritis was taking cimetidine and ketoprofen, a non-steroidal anti-inflammatory agent.

Tissue specimens

Firm tissue from the tumour mass (mastectomy specimens and excision biopsies) was wrapped in tin foil, snap frozen in liquid nitrogen and stored at $-70°C$ or over liquid nitrogen. Serial sections 5–10 μm thick (depending on the properties of the section) were cut, dried at 37°C for 30 min and stored at $-20°C$ under desiccated conditions prior to examination within 7 days. Prolonged storage at $-20°C$ appeared to have detrimental effects on certain antigens, for example, those recognised by the OKT4 and HNK-1 antibodies.

Immunohistochemical staining

Sections were fixed in acetone at room temperature for 5 min, air-dried and immersed in 20% newborn calf serum (NBCS, Flow Labs.) in Tris-HCl buffered saline (TBS; pH7.5, 0.05M Tris; 0.85% NaCl). Excess buffered NBCS was wiped away from the sections and they were incubated in the monoclonal first layer (TBS without McAb on control sections) for 60 min at 37°C in 100% relative humidity. Excess antibody was drained and the sections were washed for 3×5 min in TBS. They were then incubated in 1/250 dilution of horse radish peroxidase conjugated rabbit anti-mouse IgG (Dako) in TBS containing 6.6% normal human serum. Following 60 min incubation (37°C, 100% relative humidity) and 3×5 min washes in TBS, sections were incubated with 60 mg% diaminobenzidine. Immediately before use, the diaminobenzidine solution was filtered and 60 μl H_2O_2/100 ml substrate was added. Following incubation for 5–10 min sections were washed in TBS. The staining of those incubated with OKT4 or HNK-1 was enhanced by immersion in 1% phosphate-buffered osmium tetroxide for 2 min and subsequent washing in TBS. This did not affect the specificity of the reactions, but helped to visualise those cells the staining of which otherwise tended to be weak. Consequently, a more accurate assessment of the distribution of $HNK1^+$ cells and of T4/T8 ratios could be made. The sections were then washed in distilled water, counterstained for 5 sec, in Gill's no. 2 Haemalum, blued in hot tap water, dehydrated in a series of graded alcohols (50, 70, 95, and 100%) and cleared in xylene. Permanent mounts were made in Styrolyte (Raymond Lamb). The specificity of the antibodies was routinely controlled on sections of palatine tonsils. Staining in tissue sections treated with diaminobenzidine and hydrogen peroxide alone was routinely negligible in breast tissue.

Monoclonal antibodies

The following murine McAbs were used in this study:

2A1 IgG1 antibody identifying human HLA Class I (HLA-A, B, C) non-polymorphic determinant (Beverley, 1980).

TDR31.1 IgG1 antibody recognising human HLA Class II (HLA-DR) monomorphic determinant (DeKretser *et al.*, 1982). This reagent was supplied as purified culture supernatant (400 $\mu g\,ml^{-1}$) and used at 1/200 dilution.

MASO20 IgG1 antibody against human B cells (Clone 5/11 HLK) recognising a site close to the

monomorphic determinant of the DR molecule (Trucco *et al.*, 1979). This antibody was not identical to TDR 31.1.

2D1 IgG1 antibody directed against a human haemopoietic cell antigen (HLeI) of mol.wt. 70 K dalton present on lymphoid and myeloid cells, weakly expressed on granulocytes, monocytes and early erythroid precursors. Absent from a wide variety of epithelia (Beverley *et al.*, 1980).

UCHT1 IgG1 antibody reactive with antigen of 19 K dalton mol.wt. expressed on peripheral T cells and showing identical reactivity with the monoclonal antibody OKT3 (Kung *et al.*, 1979).

OKT4 IgG2b antibody reactive with 62 K dalton mol.wt. antigen expressed on human T cells of helper/inducer subclass (TH_2-T cells) (Kung *et al.*, 1979; Reinherz *et al.*, 1979*a, b*).

OKT8 IgG2a antibody reactive with an undefined antigen of peripheral T cells of cytotoxic/suppressor subclass (TH_2+) (Reinherz *et al.*, 1980).

OKM1 IgG2b antibody reactive with mature monocytes, granulocytes and certain circulating null cells (Breard *et al.*, 1980) including cells which mediate NK activity (Zarling & Kung, 1980) and antibody-dependent cellular cytotoxicity (Kay & Horowitz, 1980).

HNK-1 IgM antibody (Leu 7) which defines a differentiation antigen selectively expressed on NK and antibody-dependent killer (K) cells (Abo & Balch, 1981).

TDR31.1 was a gift of Drs W. and J. Bodmer, Imperial Cancer Research Fund, London and 2A1, 2D1 and UCHT1, generous gifts of Dr P.C.L. Beverley, ICRF Human Tumour Immunology Unit, University College Hospital, London. OKT4, OKT8 and OKM1 were supplied by Ortho Pharmaceutical Corporation, New Jersey, USA; MAS020 by Sera-Lab, UK and HNK-1 by Becton Dickinson Monoclonal Center, Inc., USA.

Results

Histopathological and immunocytochemical data on sections from the 16 benign and 17 malignant tissues are summarised in Tables I and II.

2A1 (anti HLA, -A, -B, -C)

Antigens recognised by this antibody which stained both the membrane and cytoplasm were detected consistently on the leucocytes, as well as endothelial cells and stromal fibroblasts of tumour tissue, but some epithelial cells of benign and malignant tissue were negative while others were positive with various degrees of staining intensity (Figure 1). In 5/16 benign tissue specimens staining was uniform but in the remaining 11 it was more variable. However, by contrast with the malignant tumours none of the benign lesions exhibited *completely* negative staining of epithelial cells with this McAb.

Uniform staining of epithelial cells was not observed in any of the 17 malignant tissue specimens. In 8, the pattern was heterogeneous (e.g. Figure 2) while the remainder were uniformly negative. There was no correlation between 2A1 and TDR31.1, 2D1 or UCHT1 staining.

TDR31.1 (anti HLA-DR)

Reactivity with antibody ranged from weak, intermittent to strong membrane and cytoplasmic staining (Figure 3) of both the cells of benign and malignant tissues.

Lymphoid and non-lymphoid cells appeared to be stained, the latter comprising a range of morphological features including benign and malignant epithelial cells, elongated cells reminiscent of dendritic cells, endothelial cells in capillaries and macrophages. The staining of epithelial cells, benign and malignant – was sporadic and never uniform.

There was no correlation between epithelial TDR31.1 and 2D1 (or UCHT1) staining. However, lymphoid cells stained with TDR31.1 although numerically fewer were positively correlated with those stained with 2D1 and UCHT1 (Tables I and II).

2D1 (anti leucocyte)

Epithelial cells in all specimens were negative for antigens detected by this antibody. The majority of positive cells possessed the rounded morphology characteristic of lymphoid cells but occasional elongated cells mostly resembling fibroblasts in the periductular stroma were also stained. Round cell staining was mainly confined to the inter-epithelial and periductal areas in benign tissues, while elongated cells were most frequently found in the stroma (Figure 4).

Round cell staining was a more frequent and prominent feature of malignant than benign tissue and not, by contrast with the latter, principally found in association with ducts. Positive cells were characteristically found in the stroma surrounding tumour foci, with relatively few actually detectable within the tumour mass (Figure 5). There appeared to be no correlation between the extent and distribution of 2DI staining and necrosis.

UCHT1 (anti pan T cell)

Staining patterns observed with UCHT1 were

Table I Summary of immunohistological data derived from serial sections of 16 benign breast tumours

Patient no.	Age	Histology	Epithelial Cell staining[b] 2A1	Epithelial Cell staining[b] TDR31.1	Inflammatory cell infiltrate staining[a] TDR31.1	2D1	UCHT1	MASO20	OKT4/8 ratio	OKM1
002	37	Adenosis	+/−	+/−	+	++	++	++	>1	−
048	22	Adenosis	+	+/−	++	++	++	+	>1	+
031	49	Adenosis	+	+/−	++	+++	++	−	>1	−
047	32	Adenosis	+	+/−	++	++	+	++	ND	−
006	32	Adenosis/fibrosis	+/−	+/−	+	++	++	+	>1	−
013	33	Adenosis/fibrosis	+/−	+/−	++	+++	++	+	>1	+
014	51	Adenosis/fibrosis	+/−	+/−	++	++	+	+	>1	+
041	45	Adenosis, fibrosis, duct ectasia	+/−	−/+	++	++	++	+	>1	+
007	65	Mild fibrosis	+/−	−/+	+	+	+	−	>1	−
042	49	Cystic mastopathy	+/−	−/+	+	+++	++	+	<1	−
023	44	Sclerosing adenosis	+/−	−/+	++	++	+	−	<1	+
036	43	Adenosis/epitheliosis	+/−	+/−	++	++	++	+	>1	+
020	59	Duct ectasia, epitheliosis,	+	+/−	+++	+++	++	++	>1	+
035	43	Fibroadenoma	+/−	+/−	+	+++	++	++	ND	−
045	32	Fibroadenoma	+	+/−	++	++	++	+	<1	+
050	27	Fibroadenoma	+/−	+/−	++	+++	++	+	>1	+

ND = Not Done.

[a]Staining reactions are scored on a semi-quantitative scale from 4+ (many cells stained) to − (no cells stained) for the inflammatory cell infiltrate, apart from OKM1 where the relative scale is from + to −.

[b]Staining reactions with anti-HLA antibodies are denoted by + (homogeneous staining); − (no staining) and +/− (heterogeneous staining).

similar in microanatomical distribution to those of 2D1 (Figures 6 and 7) such that UCHT1$^+$ cells accounted for ~75% 2D1$^+$ cells, regardless of the tissue examined. Some positive cells were noticeably larger than others and possibly corresponded to T cell blasts. 2D1 and UCHT1 staining was broadly correlated with lymphoid TDR31.1 staining (Tables I and II). Virtually none of the UCHT1$^+$ cells in the few malignant tumours also examined with HNK-1 (see below) appeared to react with the latter antibody, although certain similarities in the patterns of staining were observed in some benign lesions.

OKT4/OKT8 (anti T helper/inducer/T suppressor/ cytotoxic subsets

OKT4/OKT8 ratios exceeded unity in the majority of benign (11/14) and malignant (12/17) tissues (e.g. Figures 8 & 9), and there was no evidence of microanatomic segregation, the pattern of distribution following that of UCHT1$^+$ cells in both benign and malignant tissues.

MAS020 (anti B cell)

Relatively few cells were stained with this McAb. Benign tissues were mainly characterised by the presence of small numbers of positive interepithelial cells. In the periductal areas and in stromal tissue, occasional staining was observed which appeared to correspond to fibroblasts. Epithelial cells were negative but a proportion of endothelial cells in some sections were positive. A feature in common with OKM1 was the staining of intraluminal material.

HNK1 (anti NK/K cells)

The numbers of specimens stained with this antibody were limited. No positive cells were observed in 3 malignant tissues, two of which – 003 and 009 – had marked leucocyte infiltrates. Tissues positive for OKM1 (003 and 010) were negative for HNK1. Two of 5 benign tissues (cases 002 and 014) gave positive reactions essentially confined to the ductal and periductal regions as described for UCHT1, 2D1 and OKM1 but to a quantitatively lesser extent. Of considerable interest was the reactivity, characteristic of this antibody, observed against myoepithelial cells (Figure 10) including their cytoplasmic processes.

OKM1 (anti monocyte/large granular lymphocyte)

Relative to 2D1, cells reactive with this antibody were generally fewer in all sections examined. In benign tissues, OKM1$^+$ cells with the morphology

Table II Summary of immunohistological data derived from serial sections of 17 malignant breast tumours

Patient no.	*Age*	*Histology*	*Other features*	*Epithelial cell staining*		*Inflammatory cell infiltrate staining*					
				2A1	*TDR31.1*	*TDR31.1*	*2D1*	*UCHT1*	*MAS020*	*OKT4/8 ratio*	*OKM1*
009	75	Infiltrating lobular carcinoma		−	−	+++	+++	+++	++	<1	−
046	61	Infiltrating and *in situ* lobular carcinoma	previous mastectomy (see text)	−	−	+++	+++	++	++	>1	+
003	59	Infiltrating lobular carcinoma		−	−	++	+++	++	++	>1	+
019	67	Mucoid carcinoma	necrosis	−	−	++	+++	+++	+	>1	+
040	52	Infiltrating lobular carcinoma infiltrating and *in situ* ductal carcinoma	necrosis	+/−	+/−	++	++++	+++	+	>1	+
022	52	Intraduct carcinoma	necrosis	−	−	+	+++	+	++	>1	−
032	61	Infiltrating ductal carcinoma	necrosis	−	−	++	++	++	+	<1	+
053	51	Infiltrating ductal carcinoma		−	−	+++	++	+	++	>1	−
026	70	Infiltrating ductal carcinoma		−	−	++	++	++	+	<1	−
052	75	Infiltrating ductal carcinoma	necrosis	+/−	−	+++	++++	+++	+	>1	−
043	50	Infiltrating ductal carcinoma	necrosis	+/−	+/−	+++	+++	++	++	>1	+
039	60	Infiltrating ductal carcinoma	necrosis	+/−	(+)/−	++	+++	++	++	<1	+
049	60	Infiltrating ductal carcinoma	(no mastectomy)	+/−	−	++	++++	+++	++	>1	+
010	78	Infiltrating ductal carcinoma		−	−	++	+	+	+	>1	+
059	62	Infiltrating ductal carcinoma		+/−	−	+++	+++	+	+	~1	+
021	50	Infiltrating ductal carcinoma with lobular "cancerization"	necrosis	+/(−)	+/−	++++	++++	++++	++	>1	+
033	55	Infiltrating ductal carcinoma with lobular "cancerization"		+/−	+/−	+++	++++	+++	+	>1	−

Staining reactions are scored as described for **Table I**.

of macrophages were most obvious in and around dilated ducts (Figure 11). In malignant tumours, staining from virtually none at all to clusters of cells again resembling macrophages were almost invariably confined to the stroma (Figure 12). A further feature of this McAb was staining of intraluminal cells (? macrophages) and acellular material in ducts of benign lesions (cf. MAS020) though not all ducts in a given section were necessarily positive in this respect.

Discussion

The identification and characterisation of the various cell types involved at the host:tumour interface should ideally be carried out *in situ* under conditions where the structural integrity of the tissue is retained. Only in these circumstances can the inter-relationship between diverse cell populations be properly observed. The problem then becomes how to assign function into literally static milieu of interacting cells. Hitherto, the identification of immune cells in tissue sections has been largely limited to morphology but the recent availability of McAbs against different lymphocyte subsets offers the potential to interpret histological data in functional terms. Likewise, McAbs reactive with tumour cell membrane components may provide some insight into the extent of tumour heterogeneity and in the case of products of the MHC, have important implications for cell–cell interactions in the inductive and effector phases of the immune response.

Notwithstanding such advances, studies of this type are not without interpretative difficulties. Apart from being limited to a single time point in the natural history of the disease, the subdivision of specimens to meet the requirements of diagnostic histopathology, may introduce unavoidable sampling errors. Although reproducibility was within acceptable limits on serial sections from the same portion of tumour, there was virtually no opportunity for a direct comparison of the centre *versus* periphery.

A further problem is the identification of cells in circumstances where a determinant recognised by a given antibody is expressed by several cell types which cannot be unequivocally distinguished solely on morphological grounds. Although interpretation is to some extent assisted by the availability of other more definitive markers for cells of a given lineage, the distinction between some cell types requires the application of double-labelling techniques, which were beyond the scope of the present study.

While consistent staining of leucocytes and stromal cells was obtained with the anti-HLA-A, -B, -C, McAb (2A1), reactivity with tumour cells particularly of malignant provenance was less predictable. Focal staining indicative of heterogeneity of Class I antigen expression characterised many sections – malignant and benign and approximately one-half of the former were negative for this antibody under conditions where adjacent stromal tissue was strongly positive. In this respect our data are similar to those of Fleming *et al.* (1981) who using immunofluorescence with the McAb PA 2.6 to HLA Class I antigens, reported a high detection rate of these molecules in the ductal epithelium of non-malignant breast tissue, but marked heterogeneity in the epithelium of malignant tumours.

Selective absorption from plasma of HLA Class I antigens on to the surface of epithelial cells, or masking by circulating antibody to Class I molecules are not adequate explanations of the inter- and intra-tumour variation observed with 2A1, although subtle undefined conformational changes in the monomorphic determinant which might render it unreactive with the monoclonal antibody could conceivably occur. The data rather suggest that the expression of HLA Class I molecules in a major group of primary malignant breast tumours is reduced.

The reduction of HLA Class I antigens or their heterogeneous distribution within a neoplasm may have important biological implications e.g. for the associative recognition of tumour antigens by T cells and hence for immunosurveillance.

However, there was no apparent qualitative or quantitative correlation between leucocyte infiltration and HLA Class I antigen expression. In this report our data contrast with recent experience with dysplastic and malignant nevomelanocytes where the degree of mononuclear cell infiltration correlated with the expression of HLA (or β_2 microglobulin) on nevomelanocytes (Ruiter *et al.*, 1982).

Staining with the anti HLA-DR McAb (TDR31.1) also comprised lymphoid and non-lymphoid cells. The numbers of leucocytes stained were usually less than those stained with either 2D1 or UCHT1, suggesting that not all the tumour-associated T cells were DR^+. However, the extent of T cell activation by this criterion alone is difficult to assess since an anti HLA-DR McAb would also be expected to strain the minority ($\sim$25% leucocytes) B cell and monocyte/macrophage populations. The deployment of the Tac monoclonal antibody to the IL-2 receptor on activated T cells (Uchiyama *et al.*, 1981) might resolve the issue.

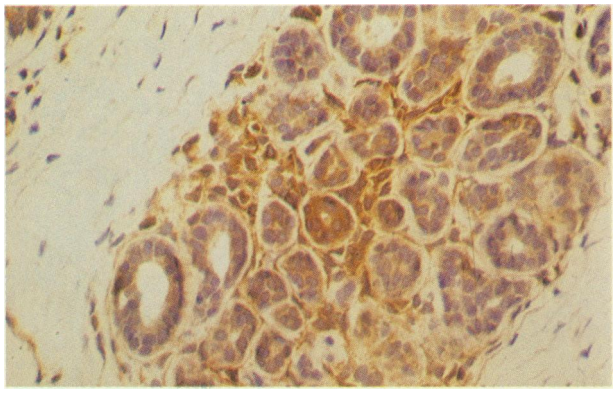

Figure 1 2A1 (anti HLA-A, -B, -C)-positive interepithelial leucocytes and periductal cells. Epithelial cell staining is variable. (Table I, Case 006, adenosis). (×170).

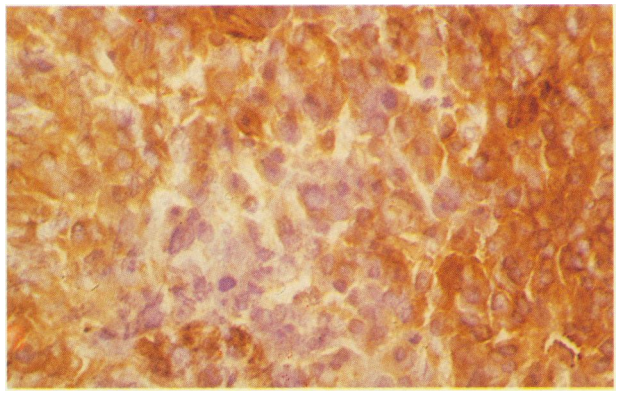

Figure 2 2A1 (anti HLA-A, -B, -C)-positive cells in an infiltrating ductal carcinoma (Table II, Case 021). The heterogeneous staining pattern is typical of approx. 50% of the malignant tumours in this series. (×170).

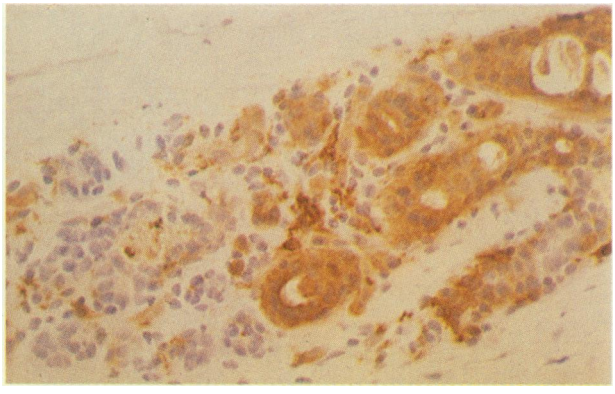

Figure 3 TDR31.1 (anti HLA-DR)-positive epithelial and periductular cells. (Table I, Case 013, adenosis/fibrosis). Note the variation in staining intensity among the ducts. (×170).

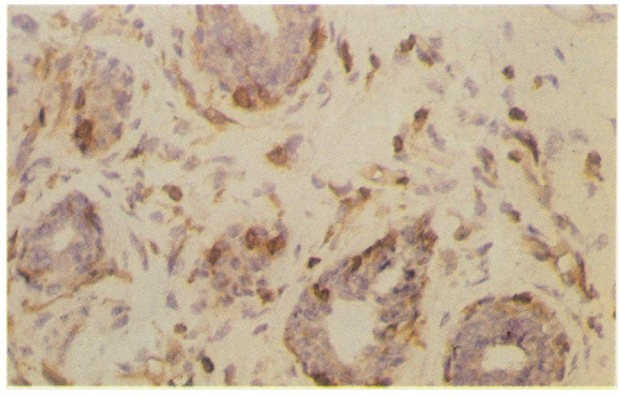

Figure 4 2D1 (anti common leucocyte)-positive interepithelial and periductal cells. Epithelial cells are negative. (Table I, Case 002). (×150).

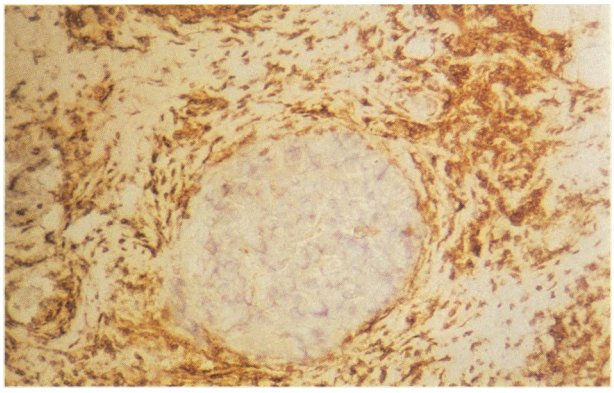

Figure 5 2D1 (anti common leucocyte)-positive round cells surrounding a duct carcinoma. (Table II, Case 021). (×70).

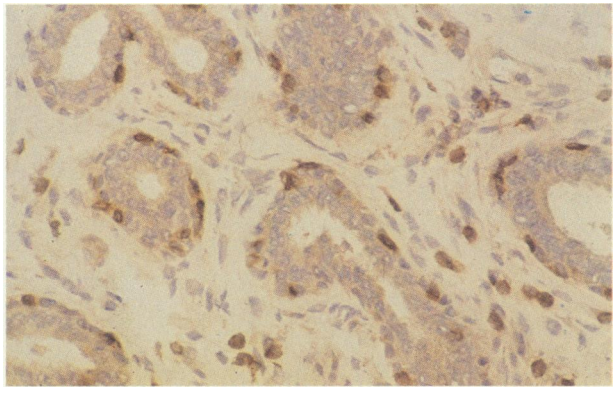

Figure 6 UCHT1 (anti pan T cell)-positive inter-epithelial and periductal cells. (Table I, Case 002, adenosis). (×170).

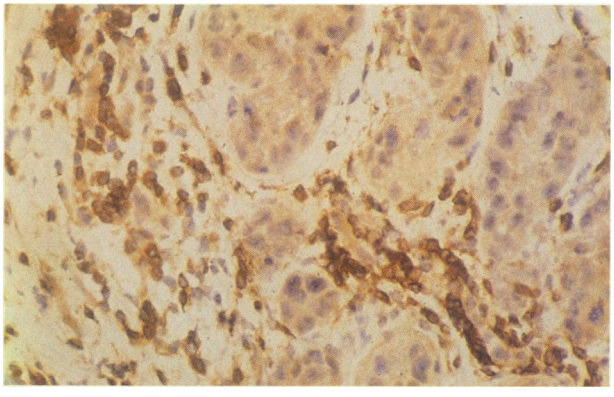

Figure 7 UCHT1 (anti pan T cell)-positive round cells surrounding duct carcinoma cells. (Table II, Case 021). (×170).

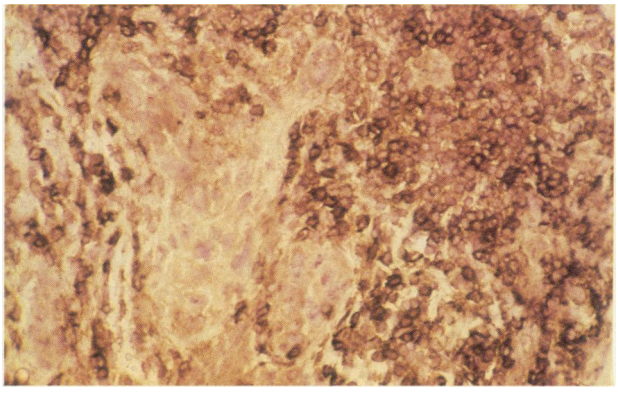

Figure 8 Preponderance of OKT4 (anti T helper)-positive cells in a duct carcinoma (Table II, Case 021). (Osmium tetroxide treated, ×170).

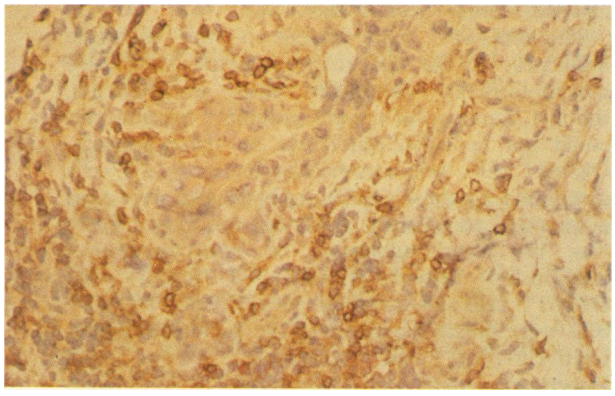

Figure 9 OKT8 (anti T cytotoxic/suppressor) cells in a duct carcinoma. Area adjacent to field in **Figure 8**. OKT8-positive cells in this tumour are fewer than those positive for OKT4 (×170).

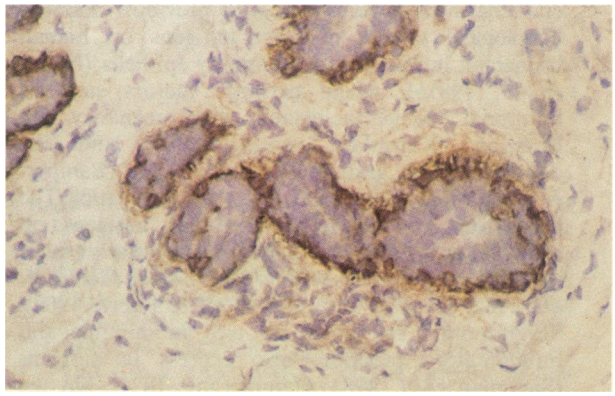

Figure 10 HNK1 (anti NK/K)-positive myoepithelial cells with very occasional positive interepithelial cells. (Table I, Case 002). (Osmium tetroxide treated, ×170).

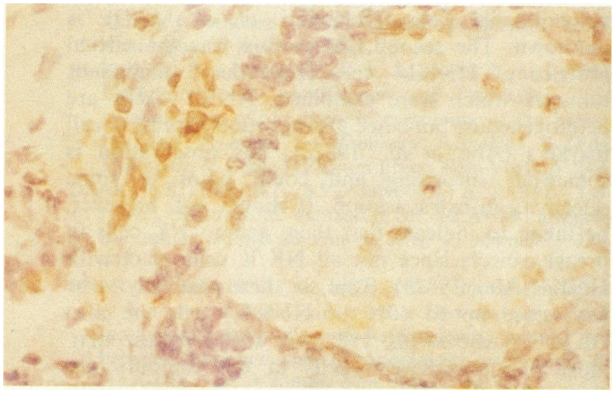

Figure 11 OKM1 (anti monocyte/LGL)-positive cells around a dilated duct of a benign lesion. (Table I, Case 041). (×170).

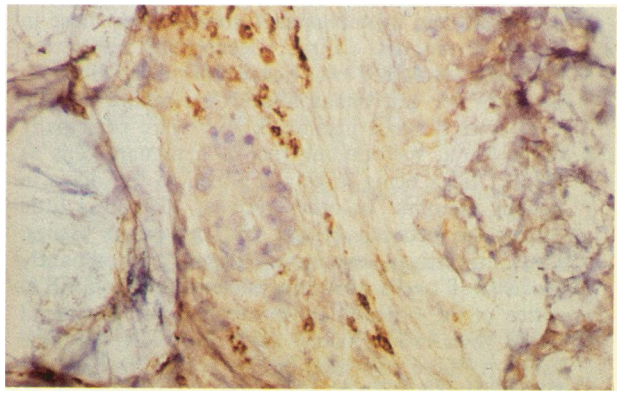

Figure 12 OKM1 (anti monocyte/LGL)-positive cells within the fibrous stroma of a mucoid carcinoma. (Table II, Case 019). (×170).

The variability of expression of HLA-DR on epithelial cells is consistent with previous immunohistological studies of these antigens on human bronchial, intestinal and mammary epithelia (Natali *et al.*, 1981*b*), where extrinsic factors such as hormonal changes associated with pregnancy and lactation (Klareskog *et al.*, 1980) and the development of graft *versus* host disease (Lampert *et al.*, 1981; Mason *et al.*, 1981) are influential. The anomalous expression of HLA-DR antigens on solid human tumours is of comparatively more recent description (Natali *et al.*, 1981*a*; Gatter *et al.*, 1982; Daar *et al.*, 1982; Daar & Fabre, 1983). Several other cell types involved in immune and inflammatory processes also express Ia antigens (Steinman *et al.*, 1981; Hammerling, 1976), but the mechanism(s) of induction is largely unknown. Since Ia antigens are important in cell:cell interactions and in antigen presentation (Lonai *et al.*, 1981), the expression of similar molecules on tumour cells could have implications for the induction of immune responses to putative tumour-associated antigens. However, why this should be a property of only some tumour cells (including those of benign origin) requires further investigation.

Staining with the antibodies 2D1, UCHT1, OKT4, OKT8 and MAS020 disclosed two features which differed in malignant tumours from those in benign tumours. First, leucocyte infiltration although variable, was generally more intense. Second, the microanatomical distribution of the leucocytes differed insofar as they were principally to be found surrounding foci of malignant cells as distinct from being largely confined to the ducts in benign tumours. In other respects there was little distinction between benign and malignant tissues; the infiltrative leucocytes were predominantly (~75%) T cells and the subset (OKT4/OKT8) ratios of the order of those reported for peripheral blood (McCluskey *et al.*, 1983). However, in some tumours there appeared to be a shift toward the suppressor/cytotoxic subset.

Other leucocytes were monitored by the monoclonal antibodies OKM1 and HNK1. Immunofluorescence flow cytometry data have shown that OKM1 is reactive with peripheral blood monocytes, granulocytes and a major proportion of circulating NK cells (large granular lymphocytes) (Ortaldo *et al.*, 1981). Although in positively stained sections, not all cells could be unequivocally identified without recourse to double-labelling techniques, macrophages appeared to be the predominant cell type. The presence of $OKM1^+$ cells in the ducts is consistent with macrophages being a component of the interepithelial leucocyte population of the human mammary gland and also accounts for the detection of similar cells in the alveolar lumina (Selig & Beer, 1981).

The $OKM1^+$ cells present in the leucocyte infiltrates of the malignant tumours were also morphologically consistent with macrophages, though staining of large granular lymphocytes could not be ruled out solely on these grounds. $OKM1^+$ cells were numerically fewer than T cells recognised by the UCHT1 antibody and were thus a minority component of all the infiltrates. There was no clear numerical or microanatomical relationship between $OKT4^+$ (T helper) and $OKM1^+$ cells. In common with T cells, $OKM1^+$ cells were mostly confined to the stromal reaction; relatively few had penetrated tumour foci. Functional data on recovered macrophages attributing them with an *in vivo* cytotoxic role should be interpreted in this awareness.

Although in this study relatively few sections were stained with the monoclonal antibody HNK1, two points of interest emerged. The positive cells in benign sections corresponded largely to ductal interepithelial leucocytes and by contrast with the other monoclonal antibodies used here, there was consistent staining of myoepithelial cells. The significance of this cross-reactivity and the relationship between HNK1 and other markers of myoepithelial cells (Bussolati *et al.*, 1983) is unknown. The second observation was the virtual absence of $HNK1^+$ cells from the 3 malignant tumours which were examined. $HNK1^+$ cells are heterogeneous, but since a proportion express T cell markers (Abo *et al.*, 1982*a*), this observation is somewhat surprising and could point to some selectivity in extravasation. It should not, however, be taken to indicate that there are no NK cells in breast cancers since not all NK/K cells react with HNK1(Abo, 1982*b*). Even so, there are likely to be few: since up to 60% of $HNK1^+$ cells are also $OKM1^+$ (Abo *et al.*, 1982a) it would appear that most of the $OKM1^+$ cells in the tumours are macrophages. This conclusion accords with functional studies conducted with lymphoid cells recovered from freshly disaggregated neoplasms wherein breast, in common with other solid tumours has generally disclosed low or non-existent levels of NK activity, (Vose *et al.*, 1977; Totterman *et al.*, 1978; Moore & Vose, 1981; Eremin *et al.*, 1981; Introna *et al.*, 1983). The recent availability of McAbs with greater selectivity for NK cells should allow further examination of this question (Perussia *et al.*, 1983*a*, *b*).

Note added in proof

Since this manuscript was submitted, similar data supporting the major findings of this study have

appeared elsewhere. *See* Bhan, A.K. & Des Marais, C.L. (1983). Immunohistologic characterization of major histocompatibility antigens of inflammatory cellular infiltrate in human breast cancer, *J. Natl Cancer Inst.*, **71**, 507.

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