ANTIGENIC HETEROGENEITY OF BREAST CELL LINES
DETECTED BY MONOCLONAL ANTIBODIES AND ITS
RELATIONSHIP WITH THE CELL CYCLE

PAUL A. W. EDWARDS1*, ROBERT A. SKILTON1, ANDREW W. R.
PAYNE2 AND MICHAEL G. ORMEROD2
1Ludwig Institute for Cancer Research (London Branch) and
2Institute of Cancer Research: Royal Cancer Hospital, The Haddow Laboratories, Clifton
Avenue, Sutton, Surrey, U.K.

SUMMARY

Established cell lines were stained by immunofluorescence with four monoclonal antibodies to
study the phenomenon of antigenic heterogeneity and its possible relation to the cell cycle. Five
cell lines thought to be of breast origin, MCF-7, ZR-75-1, T47D, MDA-MB-231 and HBL-100,
were stained with three monoclonal antibodies (LICR-LON-M8, LICR-LON-M18, LICR-LON-
M24) that each stain a distinct subset of normal breast epithelial cells, i.e. demonstrate antigenic
heterogeneity in normal breast epithelial cells. Contrasting monoclonal antibodies LICR-LON-
FIB75 and LICR-LON-FIB86 to homogeneously expressed antigens were also used.

For the antibodies demonstrating heterogeneity distinct positive and negative fractions were not
seen by flow cytometry; the intensity of fluorescence varied continuously from background to a
hundred times stronger than background. Fluorescent DNA staining showed no obvious relation
between antigen expression and the cell cycle. The essentially constant proportion of cells of a
given antigenic phenotype in the various phases suggests that these antibodies do not distinguish a
phenotype associated with a distinct proliferating population of cells.

INTRODUCTION

Several groups working with monoclonal and polyclonal antibodies that bind to
normal and neoplastic human epithelial cells have observed 'antigenic heterogeneity'
(e.g. see Arklie et al. 1981; Daar & Fabre, 1983; Edwards & Brooks, 1984; Foster,
Dinsdale, Edwards & Neville, 1982a; Foster, Edwards, Dinsdale & Neville, 1982b;
Fox, Damjanov, Knowles & Solter, 1983; Hand, Nuti, Colcher & Schlom, 1983;
Hilkens et al. 1983; Peterson, Ceriani, Blank & Osvaldo, 1983); when a monoclonal
antibody is used to stain either normal epithelium or a tumour of epithelial origin the
intensity of staining varies widely from one cell to another, and different cells stain
with different antibodies (Edwards & Brooks, 1984; Hand et al. 1983). The staining
does not appear to be related to morphology; in other words morphologically
homogeneous epithelial cells are heterogeneous in their antigen expression. The

*Present address: Department of Pathology, University of Cambridge, Tennis Court Road,
Cambridge.

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biological significance of antigenic heterogeneity is not known – it could be a trivial 'random' variation of antigen expression or the different antigens may be expressed in different stages of differentiation, for example. It has also been suggested that such antigen expression may sometimes be related to the cell cycle (Kufe et al. 1983).

Three monoclonal antibodies raised in this laboratory to human breast epithelial cells (Table 1) reveal antigenic heterogeneity in normal human breast (Edwards & Brooks, 1984; Foster et al. 1982b) and in breast tumours (Foster et al. 1982a). In the normal breast the antibodies each stain a different population of the luminal epithelial cells, even in intact unfixed tissue, and the different populations are distributed in characteristic ways, suggesting that the antigen expression may not be random (Edwards & Brooks, 1984). Several other epithelia are also stained, showing varying degrees of antigenic heterogeneity (Foster et al. 1982a; P. A. W. Edwards, unpublished observations).

Cell lines can show antigenic heterogeneity (Edwards & Brooks, 1984; Handef et al. 1983; Peterson et al. 1983) and because they divide rapidly are ideal for cell cycle analysis. We describe here the antigenic heterogeneity displayed in various cell lines thought to be of breast origin and show that it is not related in any simple way to the cell cycle, nor are particular antigens associated with a distinct proliferating fraction of the cells, if the S and G2-M phases of the cell cycle occupy a constant time.

MATERIALS AND METHODS

Monoclonal antibodies

The monoclonal antibodies to human breast epithelial cells were raised using mice immunized with human milk fat globule and have been described (Edwards & Brooks, 1984; Foster et al. 1982b). Their specificities are summarized in Table 1. Ascitic fluid was the source of antibody, and was used at a dilution of 1:100. The monoclonal antibodies LICR-LON-FIB86 and LICR-LON-FIB75 were raised similarly but using mice immunized with human breast fibroblasts (Edwards, Easty & Foster, 1980a; Edwards, Foster & McIlhinney, 1980b).

Cell lines

The breast cell lines were maintained in Dulbecco's Modified Eagle's Medium with 10% (v/v) foetal bovine serum with or without insulin and hydrocortisone as follows. Four of the cell lines used were derived from pleural effusions of breast tumours (Engel & Young, 1978). The line MCF7 (Soule et al. 1973) was obtained from the laboratory of origin and used at passage 287. Three other samples of the line were obtained from different sources at different passage numbers and gave essentially similar results except that one strain seemed to have only a few cells staining with M18, and in the two other strains no M24-positive cells were found. ZR-75-1 (Engel et al. 1978) was obtained from Dr M. Lippmann, and cultured with 8 μg/ml insulin. T47D (Keydar et al. 1979) was obtained from Dr H. Freake, Royal Postgraduate Medical School, Hammersmith, U.K. and was used at passage number about 100, cultured with 8 μg/ml insulin, 1 μg/ml hydrocortisone. MDA-MB-231 (Cailleau, Young, Olive & Reeves, 1974) was obtained from the Mason Research Institute, Rockville, Maryland, U.S.A. The cell line HBL-100 (Polanowski, Gaffney & Burke, 1976), derived from normal milk cells, was also obtained from the Mason Research Institute.

Only cultures in which no mycoplasma could be detected by staining with Hoechst dye 33258 (Chen, 1977) were used. Our stocks of the cell lines MDA-MB-231 and HBL-100 were found to be contaminated and were freed of detectable mycoplasma by treatment with high concentrations of
Table 1. The monoclonal antibodies specific for luminal epithelial cells

<table>
<thead>
<tr>
<th>Designation</th>
<th>Antigen or epitope</th>
</tr>
</thead>
<tbody>
<tr>
<td>LICR-LON-M8</td>
<td>High molecular weight glycoprotein, the EMA antigen (Ormerod, Steele, Edwards &amp; Taylor-Papadimitriou, 1984)</td>
</tr>
<tr>
<td>LICR-LON-M18</td>
<td>Galβ (1→4) GlcNAc β(1→6) (Gooi et al. 1983)</td>
</tr>
<tr>
<td>LICR-LON-M24</td>
<td>Several glycoprotein bands, predominantly a broad band of 39 to 59×10³ M, and also glycolipid. (R. A. J. McIlhinney, M. Gore &amp; S. Patel, unpublished data)</td>
</tr>
</tbody>
</table>

Within the human breast, the antibodies bind only to the luminal membrane of the epithelial cells, and only to some of these (Foster et al. 1982b). Each antibody defines a different subset of the luminal epithelial cells (Edwards & Brooks, 1984).

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antibiotics with putative anti-mycoplasma activity: Aureomycin, Lincomycin, Tylosin. The original contaminated stocks of both lines gave the same results as the treated mycoplasma-free cells.

**Immunofluorescence**

Cultures were stained essentially as described previously (Edwards, Brooks & Monaghan, 1984). The cell lines were grown to about 80% confluence on “Petriperm” dishes (Heraeus, Stockport, U.K.) were washed with “assay medium” (Dulbecco’s Modified Eagle’s Medium with 10mM HEPES replacing bicarbonate (pH 7.4), with 1% (v/v) foetal bovine serum, 1% (w/v) bovine serum albumin, plus 3mM NaN₃), cut up into 1-cm squares and transferred to 24-well tissue-culture dishes (Costar, obtained via L. H. Engineering, Stoke Poges, Bucks., U.K.). They were incubated unfixed on ice for 1 h with monoclonal antibody in assay medium at 4°C, washed three times, incubated for 1 h with fluorescent-conjugated second antibody at 4°C, washed three times and mounted in 9% (w/w) poly(vinyl alcohol) (Goshenol, Polaron Ltd, Watford, U.K.)/23% (w/w) glycerol in 50mM-Tris HCl (pH 8.5 at 20°C) (Heimer & Taylor, 1974)). IgG, IgM-specific fluorochrome-conjugated second antibodies were used for the two-colour immunofluorescence: antibody M8 is IgG, while antibody M18 is IgM. Fluorescence was generally photographed with exposures of 15 s on XP1 film (Ilford, Mobberley, Cheshire, U.K.), using a Polyvar microscope (Reichert, Vienna). Fluorescent-conjugated second antibodies were obtained and ‘cleaned up’ as described (Edwards & Brooks, 1984). Controls for fluorescence staining included substitution of an irrelevant monoclonal antibody LICR-LON-R10 (Anstee & Edwards, 1982) that does not bind to any of these cell lines (e.g. see Fig. 3f).

**Flow cytometry**

Cells were harvested in log phase of growth using EDTA for up to 10 min (some experiments with cell line T47D) or EDTA+2.5 mg/ml trypsin for 3–5 min at room temperature. If badly clumped, suspensions were then passed through a 0.4 mm needle. They were washed and stained in assay medium as for cultures, centrifuging for 5 min at 100g. A goat anti-(all mouse immunoglobulin) fluorescein conjugate was used (Nordic Immunological Labs). Cells were then centrifuged and suspended in 200 μl phosphate-buffered (pH 7.4) saline and vortexed while 5 ml of cold ethanol (4°C) were added dropwise. After 1 h at room temperature the fixed cells were centrifuged and resuspended in 1 ml 200 μg/ml RNase in phosphate-buffered saline and incubated for 10 min at 37°C; 1 ml 40 μg/ml propidium iodide was added and the suspension passed through a 0.4 mm needle before analysis.
The cells were analysed on an Ortho cytofluorograph 50H and associated computer (2150) using an argon-ion laser tuned to 488 nm. The peak and area of the red fluorescent signal (propidium iodide), area of the green fluorescent signal (fluorescein) and peak of the signal from forward scatter were recorded in list mode on the 2150 computer for a minimum of $2 \times 10^4$ cells at a flow rate of 500 cells/s. Peak red (DNA) fluorescence was displayed against area in order to distinguish fluorescence from cells in $G_0$ of the cell cycle from that from two cells in $G_1$ stuck together. These 'doublets' were then excluded from further analysis (see Fig. 3). By drawing the appropriate regions on the display of DNA fluorescence, the computer could be instructed to store in separate histograms the green (antibody) fluorescence from cells in two selected parts of the cell cycle (Fig. 3). The computer was programmed to calculate the median channel number for each of these histograms and this was used to compare the relative amounts of antibody bound to the cells in different parts of the cell cycle. The number of cells in a particular range of intensity of antibody fluorescence could also be read out from these histograms, allowing the cell cycle characteristics of cells of given antibody staining to be estimated. For an alternative method of displaying the data, the computer was programmed to calculate the median channel number for green (antibody) fluorescence, for each value (channel number) for red (DNA) fluorescence and then to plot median of green fluorescence against red fluorescence (Fig. 4).

RESULTS

Staining of monolayer cultures

Examples of immunofluorescence staining of the cell lines are shown in Figs 1 and 2. Two-colour fluorescence was used so that the heterogeneity of expression of antigens could be related. The cell lines MCF7 and ZR-75-1 stained with all three anti-epithelial cell antibodies, M8, M18 and M24. Staining varied widely in intensity between different cells of the same culture, and in several cases the different antibodies picked out distinct subsets of the cells. The most striking heterogeneity of staining was shown by the cell line MCF7 stained with antibodies M8 and M18 (Fig. 1). In contrast, some cells of the line ZR-75-1 (Fig. 2) were stained brilliantly by antibody M18 but most were at least weakly positive for staining by antibody M8 as well. Antibody M24 stained a small number of cells in both lines, some of these cells staining strongly (not shown). The cell line T47D (not shown) showed staining only by antibody M8, rather more than half the cells being stained to some extent.

The line MDA-MB-231 was stained only by antibody M18 (about 30% of the cells) and HBL100 was stained only by antibody M18, and then very weakly, requiring flow cytometry for confirmation. Incidentally, this means that neither of these lines has an antigenic phenotype expected for normal or neoplastic breast epithelial cells (Foster et al. 1982a).

The monoclonal antibodies LICR-LON-FIB75 and LICR-LON-FIB86 provided contrasting examples of staining, as they generally stain in a different way from the three anti-epithelial cell antibodies. LICR-LON-FIB75 stains most adult...
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Figs 1 and 2
Fig. 3
human cells in solid tissues rather uniformly (Buckman et al. 1982). It stained all
the cell lines uniformly and provides a useful contrast to the heterogeneity of
staining by the anti-epithelial cell antibodies, M8, M18 and M24 (see cytometry data
below). FIB86 has a more restricted specificity, and many but not all cells of
epithelial origin fail to stain with it (Edwards et al. 1980a; Gusterson, Edwards,
Foster & Neville, 1981; Edwards, unpublished observations). All of the three lines
discussed above, MCF7, ZR-75-1 and T47D, showed no clearly positive staining by
FIB86 under the microscope. In contrast, the lines MDA-MB-231 and HBL-100
both stained well, and all cells were stained relatively uniformly.

Thus the lines showed a variety of detailed staining patterns and varying degrees
of antigenic heterogeneity, from the striking heterogeneity of staining of MCF7 by
antibodies M8, M18 and M24 (Fig. 1), through the fairly but not completely general
staining of ZR-75-1 by antibody M8 (Fig. 2), to the more or less uniform staining by
antibodies FIB75 and FIB86.

Flow cytometry

For flow cytometry, the cells were stained with each antibody singly using a
fluorescein-conjugated second antibody. Their nuclei were stained with propidium
iodide so that antigen expression could be related to the position of the cells in their
cycle.

Typical results are shown in Fig. 3. As is described in Materials and Methods,
peak DNA fluorescence was displayed against its area and results from cells clumped

gether were excluded (Fig. 3A). The fluorescence data could then be displayed in
two ways – either as a cytogram of antibody fluorescence against DNA fluorescence
(Fig. 3E) or by displaying, in a histogram, the antibody fluorescence of cells in a
particular part of the cell cycle (selected by placing a region on the DNA cytogram
(Fig. 3A)); two such histograms could be displayed at one time. An example is
shown in Fig. 3 where histograms A2 and A3 (Figs 3c,d) show the antibody

Fig. 3. Flow cytometry of immunofluorescence staining by antibodies as a function of
the cell cycle, monitored by fluorescent staining of DNA. Cell line MCF7 stained by
monoclonal antibodies M8 (a–f) and FIB75 (g,h). a. ‘Cytogram’ of DNA fluorescence
signals. Each dot represents a cell, its y coordinate being the peak height of its DNA
fluorescence signal, its x coordinate being the area of its DNA fluorescence signal. The
quadrilateral labelled 1 encloses single cells. Pairs of cells appear at the same y value
(peak height) as single cells but at higher values of x (area). b. Histogram of DNA
fluorescence for single cells taken from area 1 in a, showing distribution of cells through
the cell cycle. c. Histogram of immunofluorescence (Ab fl.) of cells in Go + G1 from area
2 in a. d. Similar histogram for cells in G2 + M from area 3 in a. e. Cytogram of
immunofluorescence staining by antibody (y axis) against DNA fluorescence (x axis) for
single cells, showing the heterogeneity of fluorescence staining in the various phases of
the cell cycle. f. Cytogram, as e but for a negative control monoclonal antibody LICR-
LON-R10 that does not stain MCF7. g. Cytogram, as e but for staining by antibody
FIB75, showing its lack of heterogeneity, reflected in the smaller vertical scatter of the
points and the absence of negative cells. The expected 1–6-fold increase in fluorescence
per cell between Go+G1 and G2+M is also clear. h. Histogram of fluorescence of cells
in Go+G1 from g. Compare with histogram c.
Table 2. Median channel number for antibody fluorescence for cells in $G_0/G_1$ or $G_2/M$ of the cell cycle

<table>
<thead>
<tr>
<th>Antibody</th>
<th>$G_0/G_1$</th>
<th>$G_2/M$</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>M8</td>
<td>202</td>
<td>377</td>
<td>1.9</td>
</tr>
<tr>
<td>M18</td>
<td>23</td>
<td>37</td>
<td>(1.6)</td>
</tr>
<tr>
<td>M24</td>
<td>23</td>
<td>36</td>
<td>(1.6)</td>
</tr>
<tr>
<td>FIB75</td>
<td>129</td>
<td>218</td>
<td>1.7</td>
</tr>
<tr>
<td>None</td>
<td>25</td>
<td>40</td>
<td>(1.6)</td>
</tr>
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</table>

**T47D cells in:**

<table>
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<tr>
<th>Antibody</th>
<th>$G_0/G_1$</th>
<th>$G_2/M$</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>M8</td>
<td>89</td>
<td>187</td>
<td>2.1</td>
</tr>
<tr>
<td>M18</td>
<td>39</td>
<td>81</td>
<td>2.1</td>
</tr>
<tr>
<td>M24</td>
<td>29</td>
<td>52</td>
<td>1.8</td>
</tr>
<tr>
<td>FIB75</td>
<td>241</td>
<td>452</td>
<td>1.8</td>
</tr>
<tr>
<td>None</td>
<td>12</td>
<td>22</td>
<td>(1.8)</td>
</tr>
</tbody>
</table>

**MCF7 cells**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>$G_0/G_1$</th>
<th>$G_2/M$</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>M8</td>
<td>1</td>
<td>4</td>
<td>—</td>
</tr>
<tr>
<td>M18</td>
<td>27</td>
<td>36</td>
<td>1.3</td>
</tr>
<tr>
<td>M24</td>
<td>1</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>FIB75</td>
<td>307</td>
<td>483</td>
<td>1.6</td>
</tr>
<tr>
<td>None</td>
<td>1</td>
<td>4</td>
<td>—</td>
</tr>
</tbody>
</table>

**MDA-MB-231 cells**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>$G_0/G_1$</th>
<th>$G_2/M$</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>M8</td>
<td>350</td>
<td>507</td>
<td>1.4</td>
</tr>
<tr>
<td>FIB75</td>
<td>126</td>
<td>187</td>
<td>1.5</td>
</tr>
<tr>
<td>None</td>
<td>10</td>
<td>17</td>
<td>(1.7)</td>
</tr>
</tbody>
</table>

**ZR-75-1 cells**

N.B. The experiment for each cell line was run on a different day. Median channel number reflects the relative amount of antibody bound to a particular cell line but comparison between cell lines cannot be made as different gain settings were used.

The results are summarized in Table 2. The ratio between the median channel numbers (reflecting the amount of antibody bound to the cells) in the two parts of the cell cycle was close to that expected from the increase in surface area as the cells progressed through their cycle. During a complete cycle total surface fluorescence must double but part of the increase will occur in $G_0/G_1$. Variation in the ratio of surface area to volume of the cells through the cycle can be adjusted by the formation of microvilli (Pasternak, 1981). The Table compares cells in $G_0/G_1$ and $G_2/M$; this ratio was in the range 1.3 to 2.1, i.e. around that expected. The cell line MCF7 demonstrated higher ratios than the others, but in view of the wide spread of fluorescence for cells in $G_1/G_0$ and $G_2/M$ of the cell cycle, respectively. The median channel number for the antibody fluorescence in these two parts of the cell cycle could then be calculated.
fluorescence values, this difference was not considered to be significant. Fig. 4, in which the median channel number for green fluorescence is plotted against red fluorescence, shows the relation between the two through the cell cycle for M8 and FIB75 on MCF7 cells. The DNA contents of cells in $G_0/G_1$ and those in $G_2/M$ are marked by the arrows. Thus, there was no indication that the expression of any of the antigens was unusual in the $S$ or $G_2 + M$ phase and it can be concluded that any variation in antigen expression through the cell cycle is small compared to other sources of heterogeneity.

![Diagram](image)

**Fig. 4.** Median channel number for green (antibody) fluorescence plotted against red (DNA) fluorescence to show the expression of an antigen through the cell cycle. MCF7 cells stained with monoclonal antibodies LICR-LON-M8 and FIB75.
The most striking feature of the results was the broad distribution of the antibody fluorescence, which contrasted with the narrow distribution of the DNA fluorescence in $G_0/G_1$ or $G_2/M$ of the cell cycle. Using a cell line/antibody combination which gave strong fluorescence (M8 on T47D, for example), the staining of individual cells varied from almost negative to highly positive with a hundredfold difference in staining intensity. In contrast, the staining with the FIB antibodies revealed far less heterogeneity. This is demonstrated in Fig. 3 (G,H).

**DISCUSSION**

When the antibodies, LICR-LON-M8, LICR-LON-M18 and LICR-LON-M24, reacted with cell lines of reputed breast origin, the staining pattern revealed was invariably heterogeneous. These results confirm that the cells of such lines do not each carry a uniform set of antigens that was expressed by the tumour cell from which they were derived. Similar antigenic heterogeneity has been described using these and other monoclonal and polyclonal antisera for breast tumour cell lines (Hand et al. 1983; Peterson et al. 1983), intact breast tumours (Foster et al. 1982a; Hand et al. 1983; Hilkens et al. 1983) and other carcinomas (e.g. see Daar & Fabre, 1983; Fox et al. 1983; McGee et al. 1982). Elsewhere it has been shown that antigenic heterogeneity is also a property of normal cells: normal breast epithelial cells stained heterogeneously both in the intact, unfixed tissue (Edwards & Brooks, 1984) and in culture (Chang & Taylor-Papadimitriou, 1983; Edwards et al. 1984; Peterson et al. 1983) with these antibodies (M8, M18 and M24) and others.

The flow cytometric data clearly eliminate cell-cycle dependence as a major source of the antigenic heterogeneity observed with our antibodies. Abundance of an antigen could vary 100-fold between cells whether they were in $G_0+G_1$ or $G_2+M$; there was nothing like this magnitude of increase in antigen expression between these phases and the small increase observed is expected from the required increase in membrane area through the cycle. This was consistent with the result of Burchiel et al. (1982), who found a small increase in antigen expression through the cell cycle using monoclonal antibodies to melanoma cell lines. They suggested the increase could be due to increased membrane areas, though no figure was given. Also, Taupier et al. (1983) found heterogeneity in expression of surface immunoglobulin in a hybridoma to be cycle-independent. On the other hand, Kufe et al. (1983) presented evidence that two of the antigens defined by their monoclonal antibodies to breast tumour cells were preferentially or almost exclusively expressed during $S$ phase of the cell cycle.

Not only was the heterogeneity of antigen expression constant during the cell cycle, for our antibodies, but the data also strongly suggested that none of the antigens was associated with a distinct proliferating population of cells. It is generally expected that among similar cells the time taken in $S$ and $G_2-M$ phases is fairly constant, and differences between proliferating and quiescent populations of cells would be seen as variations in the length of $G_0-G_1$ phase, and hence in the proportion of cells in $S+G_2+M$ at any point in time. We found no evidence that
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brightly stained and negative cell populations showed a difference in the proportion of cells in $G_2+M$, for any of the antibody/cell line combinations tested (Table 2). Such a difference should have shown up in the median fluorescence values for the different phases (Table 2), and its absence was confirmed by directly measuring the proportion of cells in $G_2+M$ for brightly stained and weakly stained cells, allowing for the increase in surface area during the cell cycle. The proportion was found to be constant certainly to well within a twofold difference. Thus, for example, expression of these antigens is not associated with terminal differentiation.

As the photographs suggested and the flow cytometry demonstrated quantitatively, the heterogeneity was not merely a matter of positive and negative: there was but one population of cells with a broad and continuous distribution of fluorescence intensity from negative to widely varying degrees of positivity. This contrasted sharply with the antigen recognized by monoclonal antibody FIB75 (compare Fig. 3ε with c, and c with h) and with antigens recognized by monoclonal antibodies in, for example, the haemopoietic system: when lymphocytes are stained with subset markers such as OKT4 and OKT8, some cells are negative and some are positive, the positivity being within a limited range, so that by cytometry positive and negative fractions can clearly be distinguished. The negative cells seem to be a distinct type of cell. Leukaemia and haemopoietic cell lines, as far as we are aware, are either positive or negative for such antigens and the positive fractions show quantitatively much less heterogeneity (e.g., see Greaves et al. 1981; Taupier et al. 1983).

Are these variations in antigenic expression related to differences in the physiological state of the cell, or do cells randomly permute these molecules on their surface? Since these antibodies recognize carbohydrate structures (Table 1), such random permutation of antigens could be restricted to glycosylation. Antibodies such as FIB75 and OKT4 may be to protein epitopes and hence not show heterogeneity (for further discussion, see Edwards, 1985).

Antigenic heterogeneity has practical implications. Antibodies to antigens that are expressed by only some cells in a tumour will presumably be of limited use for targetting to tumours in vivo. One hope for bypassing this problem was that particular antigens would be expressed by proliferating tumour cells (Edwards & Foster, 1984). Our results do not encourage this idea.

We thank Isobel Brooks and Marian Barnacle for assistance; the donors listed in Materials and Methods for the breast cell lines; Dr R. A. J. McIlhinney for permission to quote unpublished work on antigen structures; Dr M. J. O'Hare for advice and help with the handling of the cell lines; and Professor A. M. Neville for advice and support. M.G.O. and A.W.R.P. were supported by a programme grant from the Medical Research Council and Cancer Research Campaign who also generously donated the Ortho Cytofluorograph to the Institute of Cancer Research.
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