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Immunoglobulin expression and synthesis by human haemic cell lines

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Summary. Twenty-six human cell lines derived from a variety of lymphoid and non-lymphoid malignancies, were investigated for their immunological markers, with special reference to the class of immunoglobulin expressed. Twenty-five of the lines stained positively for surface immunoglobulin and IgD together with IgM proved to be the major immunoglobulin classes on these cells. Six of the lines were chosen for a study of their immunoglobulin synthesis patterns over an 18-h period and the immunoglobulin produced was analysed on SDS-polyacrylamide gel electrophoresis. Patterns obtained from the cell lines were similar to that from normal lymph node lymphocytes and differed markedly to plasma cells. Two of the cell lines had abnormal immunoglobulin synthesis patterns characterized as free light chains in one case. The cell lines are evaluated for their usefulness as models of immunoglobulin synthesis and analogues of normal and neoplastic states.

INTRODUCTION

The majority of human cell lines derived from various malignant or normal sources appear to represent an Epstein–Barr virus infected blastoid population (Nilsson & Ponten, 1975; Belpomme, Minowada & Moore, 1972). Exceptions to this include cell lines derived from Burkitt lymphoma tissue and from T-cell neoplasms (Nilsson & Ponten, 1975; Kaplan, Shope & Peterson, 1974; Minowada, Ohnuma & Moore, 1972). We wish to describe twenty-six cell lines derived from peripheral blood and bone marrow of patients with a variety of haematological malignancies.

The main purpose of this investigation was to evaluate the capacity of these human cell lines to express various classes of immunoglobulin at their cell surface and their ability to synthesize immunoglobulin. Other cell markers including the receptors for the Fc component of IgG (Fcγ), the third component of complement (C3) and sheep red cells were also investigated in these populations. The possibility that cell lines can be used as models reflecting neoplastic situations *in vivo* is discussed.

MATERIALS AND METHODS

Cells

The morphological and cytochemical characteristics of twenty-five of the twenty-six cell lines have been described in a previous publication (Karpas, Hayhoe, Greenberger, Barker, Cawley, Lowenthal & Moloney, 1977). Leucocytes were isolated from patients with a variety of haematological disorders as previously described (Karpas *et al.*, 1977). All

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lines were grown for at least 6 months and many for up to 2 years before investigation. Except for line 45 all lines contained Epstein–Barr nuclear antigen-positive cells.

Plasma cells prepared from the bone marrow of a patient with IgG myeloma, and preparations of normal blood, lymph node and spleen lymphoid cells were also investigated. All these cells were prepared after Ficoll-Triosil separation as previously described (Payne, Jones, Haegert, Smith & Wright, 1976).

Cell receptors

Rosette tests for the identification of cells with receptors for Fcγ, the C3 component of complement and for sheep erythrocytes (E) have been described fully elsewhere (Haegert, Hallberg & Coombs, 1974; Smith & Haegert, 1974; Payne *et al.*, 1976). Cell suspensions were stained directly with fluorescein-conjugated rabbit or sheep antiserum to immunoglobulin heavy and light chains. Cell smears fixed in methanol and washed in saline were also stained with these antisera by the direct method. Cell suspensions and cell smears were stained with normal rabbit and/or normal sheep sera conjugated with fluorescein in control experiments. The fluorescein-labelled preparations were examined using a Leitz Orthoplan microscope fitted with a HB 200 mercury vapour Ploem illuminator.

Biosynthetic Studies

(a) *Biosynthetic labelling of cells.* Cells were incubated in RPMI 1640 without L-leucine and supplemented with 10 per cent heat-inactivated dialysed foetal calf serum and 1 per cent non-essential amino acids at a concentration of 5×10^6/ml. 10 μCi of L-(4,5^{3}H)-leucine (58 CimM) were added per 10^7 cells and the cultures were incubated in a moist 5 per cent CO_2 atmosphere at 37° for 18 h.

Viability was determined before and after the 18-h incubation by the trypan blue dye exclusion test. The cells were separated by centrifugation and lysed by disruption in phosphate-buffered saline containing 1 per cent Nonidet P40 and protease inhibitors (iodoacetamide (0·25 M) and soybean trypsin inhibitor (50 μg/ml)). Lysates and extracellular fluids were dialysed for 18 h at 4° against PBS after centrifugation for 1 h at 35,000 *g* to remove cell debris.

(b) *Detection and quantification of labelled Ig.* Synthesized immunoglobulin was quantitated by the sandwich technique of immunoprecipitation. 5 μl of a sheep antiserum with specificity to human Ig was added to aliquots of both lysate and extracellular media for 30 min at 0°. One hundred microlitres of rabbit antiserum to sheep IgG was then added to precipitate the first antibody and the tubes were left for 16 h at 0°. These conditions were previously determined to give maximal precipitation of labelled Ig. Normal sheep serum, passed down a Sepharose 4B column coupled to human Ig to remove any anti-human Ig activity, was used as first antibody in control preparations to assess co-precipitation of non-specific material.

The precipitates were washed four times by sonication in PBS containing 1 mg/ml unlabelled leucine and 0·1 per cent NP40 before counting. The total amount of radioactivity incorporated was determined by precipitation of the culture fluids with cold 10 per cent TCA.

(c) *Characterization of Ig.* Immune complexes from both specific and non-specific precipitations were dissolved in 8 M urea with 0·1 per cent SDS. After reduction and alkylation with dithiothreitol and iodoacetamide respectively, they were run concurrently with reduced and alkylated ^{125}I labelled IgM and IgG markers on 7·5 per cent cross-linked polyacrylamide, sodium dodecyl sulphate gels, 95 mm in length, which were subsequently cut into eighty slices and the amount of radioactivity in each slice determined (Maizel, 1966; Choules & Zimm, 1965).

RESULTS

(a) Immunological markers

Twenty-five of the twenty-six lines stained for surface immunoglobulin (SIg) (Table 1). The SIg-negative line contained a small percentage of cells forming spontaneous rosettes with sheep red blood cells. Surface Ig was detected on all cells in more than 50 per cent of the lines while in the remainder the SIg staining varied from 10–75 per cent. Only one light chain class was found to be present in a given culture, except in line 169.

Eighteen of the lines stained for both the M and D heavy chain class; one culture expressed M exclusively (line 126). Both M and D heavy chains

Table 1. Cell line immunoglobulin and receptor analysis

		Per cent cells with markers				
		Immunoglobulin markers		Other surface markers		
Cell line	Original* disorder	Surface Ig	Intracellular Ig	E	Fcγ	C3
104	AML	100 MDκ	0	0	0	75
118	AML	100 MDκ	5 Mκ	0	25	50
119	AML	50 Gκ	20 Gκ	0	0	50
122	AML	20MDκ, 20Gκ	2 Gκ	0	5	100
129	AML	100 Gκ	0	0	0	30
145	AML	100 MDκ	0	0	0	75
150	AML	100 MDλ	0	0	0	50
163	AML	65 MDκ	15 Mκ	0	0	0
176	AML	100 MDκ	0	0	0	5
130	AMML	100 MDκ	0	0	25	50
135	AMML	100 MDκ	10 Mκ	0	0	75
139	AMML	75 MDλ	10 Mλ	0	0	60
186	AMML	60 MDκ	10 Mκ	n.d.	n.d.	n.d.
45	ALL	0	0	5	0	0
111	ALL	20 Gλ	5 Gλ	0	0	10
155	ALL	100 MDκ	0	0	90	90
160	ALL	100 MDλ	20 Mλ	0	25	50
53	AGL	50 Gκ	0	0	0	75
107	AGL	10 Gλ	30 Gλ	0	10	40
112	AGL	100 MDλ	5 Mλ	0	15	90
132	AGL	25 Gκ	0	0	5	40
153	AGL	100 MDκ	0	0	100	100
†169 (a)	CLL	75Aλ, 5Mκ	0	0	35	40
(b)		2Aλ, 10MDκ	10 Mκ	0	30	35
173	Sezary	100 MDκ	2 Mκ	0	0	0
164	CML	50MDλ, 10G	0	0	0	60
126	HMR	50 Mκ	0	0	50	75

* AML = Acute myeloid leukaemia; AMML = acute myeloid monocytic leukaemia; ALL = acute lymphoblastic leukaemia; AGL = acute granulocytic leukaemia; CLL = chronic lymphocytic leukaemia; CML = chronic myeloid leukaemia; HMR = histiocytic medullary reticulosis.

† (a) Investigated after 3-month establishment; (b) investigated after 1-year establishment.

were always present on the same number of cells within a given culture. The intensity of staining between the two varied, M being greater than or equal to D in most cases. In lines 150, 155 and 186, D was stronger than M. Six lines stained for surface IgG immunoglobulin. A small percentage of IgG bearing cells were also present in two lines (122, 164) that stained for M and D. One line (169) contained IgAλ and IgM, IgDκ bearing cells. Many of the lines that expressed SIg also had a small percentage of cells with detectable cytoplasmic Ig (Table 1). The intracellular Ig class always correlated with that of the surface.

None of the cells in the SIg positive lines rosetted with sheep erythrocytes. In twenty-two of the lines 5–100 per cent of the cells reacted in the receptor assay for C3. A variable proportion of cells from some of these C3 positive lines had receptors for Fcγ.

(b) Synthetic studies

Six lines (104, 129, 135, 139, 160 and 169) were chosen for a study of their pattern of Ig synthesis over an 18-h period (Table 2). Synthesis studies of an IgG myeloma plasma cell preparation and lymphoid preparations from normal blood, lymph node and spleen are included in Table 2. Immunoglobulin production is expressed as a percentage of all protein synthesized and as a ratio of amounts

Table 2. Immunoglobulin synthesis by normal lymphoid preparations and cell lines

Sample	Ig synthesised as per cent of all protein	Ig Supernatant: Ig lysate	Per cent cells with intracellular Ig
Peripheral blood (1)	1·6	1·5	0
Peripheral blood (2)	1·5	1·0	0
Lymph node	2·3	0·6	0·1
Spleen	4·5	3·3	0·2
IgG plasma cells	53·0	8·3	50·0
Cell line: 104	1·2	0·3	0
129	0·8	0·3	0
135	3·2	2·3	10
139	2·4	1·9	10
160	1·4	0·8	20
169	1·8	1·6	10

found in supernatants and lysates after 18 h. The number of cells detected with intracellular Ig in the final culture preparation is also included.

All precipitations were performed in duplicate and nonspecific precipitations were 20 per cent or less of specifics.

(c) **Gel analysis**

The lysates and supernatants from the cell preparations in Table 2 were analysed using 7·5 per cent polyacrylamide gel electrophoresis. The IgG myeloma plasma cell preparation gave an expected large peak in the γ and light chain positions, the amount of radioactivity incorporated in the heavy chain compared to light approximating the expected 2:1 ratio. The blood, spleen and lymph node preparations gave peaks in the μ, γ and light chain positions on the gels. The heavy chain incorporation gave the expected 2:1 ratio to light chain.

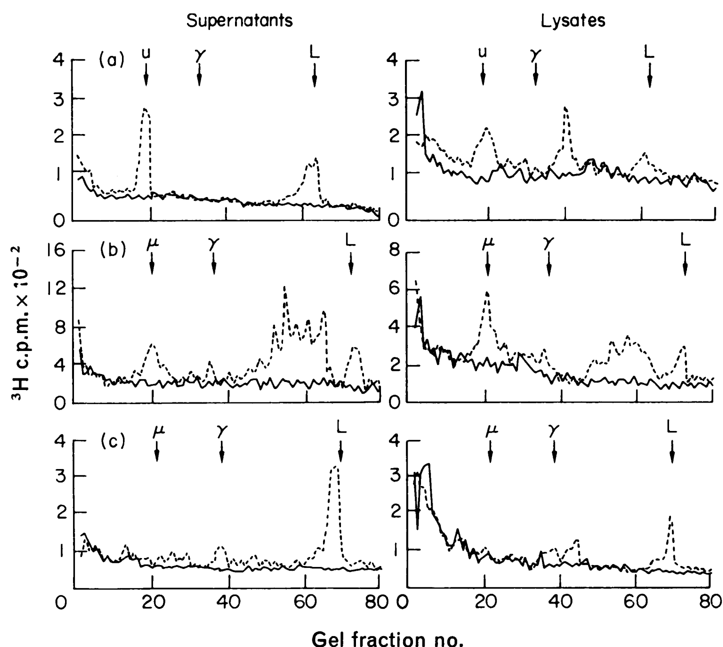

Figure 1. Polyacrylamide gel electrophoresis of fully reduced and alkylated samples. (a) Cell line no. 169; (b) cell line no. 135; (c) cell line no. 129. (- - - - -) Specific precipitations, (——) non-specific precipitations.

Four of the cell lines (104, 139, 160, 169) gave gel patterns predicted from their surface and intracellular immunoglobulin staining patterns, showing peaks in the μ and light chain positions all similar to the gel pattern for line 169 (Fig. 1a). No detectable IgA was made by line 169. (The cells cultured correspond to 169b in Table 1.) A peak running just faster than the γ marker appeared in three of the cell lysates (104, 139, 169) (Fig. 1a). Two lines showed abnormal patterns: line 129 giving a very small peak in the γ region and a large peak in the light chain position (Fig. 1c) and line 135 showing a typical μ peak and a very broad peak running just slower than the light chain peak (Fig. 1b).

DISCUSSION

All the EB virus positive cell lines studied displayed cell surface characteristics and behaviour similar to the blastoid lines derived from lymphoid origin. Only one of the lines (126), derived from a histiocytic medullary reticulosis, expressed surface IgM alone. Whether this was related to the cells involved in the disease is not known. In the remaining SIg positive lines surface IgM occurred with IgD suggesting that cells found in these cultures have some similarity to the relatively immature B lymphocytes present in chronic lymphocytic leukaemia (Fu, Winchester & Kunkel, 1974; Kubo, Grey & Pirofsky, 1974). Although IgD proved to be a major SIg, no intracellular IgD could be detected. This finding correlates to normal and neoplastic states where IgD plasma cells are rare, but lymphocytes with surface IgD are abundant (Rowe, Hug, Forni & Pernis, 1973). Detectable cytoplasmic IgM was observed in several lines with surface IgM, IgD. Comparable findings have been reported in Waldenström's macroglobulinaemia (Preud'homme & Seligmann, 1972) and in chronic lymphocytic leukaemia (Cawley, Smith, Goldstone, Emmines, Hamblin & Hough, 1976).

IgM occurring with IgD appears to be the predominant SIg expressed by cultured cells. This population emerges irrespective of the source of the culture. In line 169 the original cells (IgAλ) detectable within the first few months of culture became fewer in number as the culture period extended. This was accompanied by the detection of an MDκ line for the first time and cells containing intracellular Ig. The possibility that the original cells are persisting as non-expressors of SIg cannot be excluded.

We were unable to correlate the surface expression of IgM, IgD, IgG and intracellular Ig with the culture period of the cell lines. Intracellular Ig was seen both in early and long established cell lines. In some lines SIgG-bearing cells occurred with IgM and IgD bearers. Although extensive cytological pleomorphism was exhibited in all the cell lines investigated, the cells within a line stained homogeneously for either κ or λ light chain. The intensity of staining varied from strong to weak and the differences between the strength of IgM and IgD staining may be analagous to lymphocytes at varying stages of B-cell maturation (Preud'homme, Brouet, Clauvel & Seligmann, 1974). 'Null' cells were present in some cell cultures. These cells without detectable SIg may constitute another cell line coexisting in these cultures or simply cells with low amounts of SIg below detection by our methods. The possibility also exists that these SIg negative cells represent a subpopulation which has not yet acquired SIg—a situation analogous to a B-cell differentiation stage of which some cases of CLL are an example.

The C3 receptor was widely distributed on the cell lines, while the receptor for Fcγ was present on a minority. The expression of these receptors did not correlate with SIg class, origin of the cell line or intracellular Ig. Nevertheless, cells bearing receptors for C3 and Fcγ were clearly on one morphological cell type, often the larger cells within a line, which may suggest an association of these surface markers with different stages of cell development.

The cell lines varied in their capacity to make immunoglobulin. Some of the lines were similar to normal peripheral blood and others to lymph nodes and splenic lymphoid suspensions (Table 2). None was similar to the IgG myeloma cell preparation. Although the extracellular/intracellular Ig ratio did vary between the lines and relates to a model of B-cell maturation (Andersson, Buxbaum, Citronbaum, Douglas, Forni, Melchers, Pernis & Scott, 1974), this ratio did not correlate with cell line intracellular Ig. This pattern of intracellular staining resembled some patterns seen in CLL (unpublished observations) and was different from plasma cells. The exclusive staining of the intracellular Ig for IgM and the presence of surface IgM, IgD on the cell lines studied resembles CLL (Cawley *et al.*, 1976), and makes it unlikely that this is ingested material.

Synthesized Ig from four of the six cell lines studied gave expected gel profiles in relation to the Ig class expressed at their cell surface. IgD synthesis was not detected although this could not be excluded on the basis of its reported similar mobility to IgM (Finkelman, van Boxel, Asofsky & Paul, 1976). No IgA could be detected in line 169 which provides further evidence for the outgrowth of an IgM (IgD) population in this culture. In three of these cell lines a peak running just faster than γ was present. This peak is present in the analysis of Ig synthesis by normal mouse tissue (Andersson, Lafleur & Melchers, 1974; Vitteta, Grundke-Iqbal, Holmes & Uhr, 1974). It has been suggested that this is the Fcγ receptor but there was no correlation between the appearance or size of this peak on the gels with the expression of surface Fcγ receptor by these cell lines. In only one of these lines (169) were Fcγ receptor cells detected. We have also found this peak in Ig analysis of normal peripheral blood and CLL lymphocyte cultures.

Two of the cell lines (129 and 135) showed an unexpected pattern on gel analysis. Cell line 129, although still producing γ heavy chain, as detected by surface fluorescence and indicated on gel analysis, has a large excess of light chain being synthesized (Fig. 1c). The reported frequency of excess light chain production from human lymphoblastoid cell lines is rare (Nilsson & Ponten, 1975). Bence–Jones protein occurs in myeloma and occasionally in lymphoma and chronic lymphocytic leukaemia. We have observed by synthesis studies excess light chain production at the cellular level in CLL, lymphoma and in the L_2C guinea-pig leukaemia (unpublished observation). The exact character of the broad peak in line 135 is unclear although it may represent deleted heavy chain well documented for mouse myeloma cell lines (Birshtein, Preud'homme & Scharff, 1974) and clinically occurring as heavy chain disease.

Our findings show that cell lines derived from a variety of haematological malignancies, contain cell types capable of expressing and synthesizing Ig of various classes. These patterns of Ig expression and synthesis have analogues in normal and neoplastic situations. Some of the synthesis findings have precise counterparts in well-documented neoplastic situations. Ig synthesis by human lymphoid cell lines promises to prove useful as a model for normal and neoplastic cell study.

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Note added in proof

The T-cell line (45) did not synthesize any detectable immunoglobulin.

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