Analysis of Human Leukaemic Cells Using Cell Surface Binding Probes and the Fluorescence Activated Cell Sorter

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Abbreviations used

AUL	: Acute Undifferentiated Leukaemia
ALL	: Acute Lymphoblastic Leukaemia
AML	: Acute Myeloblastic Leukaemia
AMML	: Acute Myelo-Monocytic Leukaemia
AMonL	: Acute Monocytic Leukaemia
CML	: Chronic Myeloid Leukaemia
CLL	: Chronic Lymphocytic Leukaemia
Ph^1	: Philadelphia chromosome

PHA	: Phytohaemagglutinin
T	: Thymus-derived lymphocyte
B	: 'Bursa equivalent' derived lymphocyte
FACS	: Fluorescence Activated Cell Sorter
G_{M_1}	: Monosialoganglioside (a charged glycolipid of known structure)
SmIg	: Surface membrane immunoglobulin

This paper is dedicated to our colleague Professor G. Hamilton- Fairley who was tragically and savagely killed by a terrorist bomb in London on October 23, 1975.

Summary

Cell surface binding fluorescent ligands have been used to distinguish between different types of leukaemic cells and between leukaemic cells and their presumed normal counterparts or progenitors. Binding of these probes was evaluated using the Fluorescence Activated Cell Sorter (FACS) which provides both rapid, objective and quantitative recording of fluorescent signals from individual cells plus physical separation of cells of particular interest. Binding sites for cholera toxin (monosialoganglioside G_{M_1}) were found to be normally expressed in chronic leukaemias but greatly diminished or absent in acute leukaemias irrespective of their morphological type. Antibodies specific for the common form of acute lymphoblastic leukaemia (ALL, non-T, non-B) have been produced in rabbits. After extensive absorption and testing these were shown to define a cell surface antigen of non-T, non-B type ALLs. The antigen is absent from other leukaemias with two interesting exceptions – the majority of acute undifferentiated leukaemias express the antigen as do a proportion of chronic granulocytic leukaemias in blast crisis relapse.

The anti-ALL antibodies can therefore be used to distinguish different leukaemias and, more significantly, can identify the existence of relatively rare leukaemic cells in the blood of untreated patients and the marrow of treated patients considered to be in remission.

Introduction

Human leukaemia is a monoclonal proliferative disease (1). The leukaemias as a group are recognised as being heterogeneous, reflecting in part the cellular heterogeneity of the haemopoeitic system itself and the potential range of 'target' cells for the malignant process. The cellular diversity of disease is not surprisingly associated with a great variation in prognosis. In view of the corresponding range of therapeutic protocols available, it is clearly of great importance to establish the correct diagnosis.

Acute leukaemias still pose a considerable problem of classification. Whilst it is relatively easy to subdivide this group into myeloid and non-myeloid types, subgroupings are difficult if not impossible by morphological and histochemical criteria. Cell surface markers now provide a new and potentially more discriminating set of probes for cellular identity in leukaemia.

The philosophy and technology behind this approach has been reviewed recently (2). Cell surface phenotyping can be regarded as a form of molecular morphology with the capacity to reveal the existence of 'silent' structures on cell surfaces which can thereby serve as convenient identity tags.

Lymphoid malignancies have been extensively analysed for cell surface differentiation antigens and receptors which are characteristic of different populations or subsets of normal lymphocytes (2–4) or monocytes (5). This has led to a greater appreciation of the likely target cells involved in leukaemic processes. For example, chronic lymphocytic leukaemia is, with few exceptions, a B lymphocyte neoplasm (6) as is nodular or follicular lymphoma (7). In contrast, virtually every cutaneous lymphoma (e.g. Sezary syndrome, mycosis fungoides) appears to involve T lymphocyte derivatives (8). Acute Lymphoblastic Leukaemias appear by surface marker criteria to involve at least three separate cell types (2, 3) 70–75 % are non-T, non-B-like, 20–25 % are T cell-like, and rare cases (1–3 %) are B cell-like (cf. Burkitt's Lymphoma – ref. 9). There is suggestive evidence that those with a T cell surface phenotype have a poorer prognosis (10, 11). They usually present with higher white cell counts in the blood and in our experience are predominantly males (14 out of 15 cases). We have recently explored the potential use of two additional cell surface markers which may be particularly revealing.

We have used the binding of cholera toxin to its natural 'receptor' – Monosialoganglioside G_{M1} (12) as a probe for defective cell membrane glycolipid in leukaemias. The rationale of this approach is based upon the extensive evidence that transformation of animal cells by viruses or carcinogens is usually associated with pronounced simplification of membrane glycolipids including gangliosides (13). Our results suggest that cholera toxin may provide an extremely useful indicator of acute leukaemic cells.

The second type of marker system employs an antiserum specific for a particular type of leukaemia. There is a long and somewhat tortuous history of attempts to produce such reagents and it is only very recently that some success has been achieved (14, 15, reviewed in 2). We have raised antisera in rabbits to the non-T, non-B or common form of ALL. After extensive absorption the sera can be used to identify ALL cells.

Three key features of this analysis are (1) the availability of chemically homogenous markers with well defined specificity; (2) fluorescent labelling of probes of marker antibodies to permit identification of individual cells; (3) evaluation of reactivity using the Fluorescence Activated Cell Sorter or FACS (2). All the fluorescence reactions on living cells we carry out can, in fact, be reasonably well analysed by conventional methods (e. g. ultra-violet microscopy with plume or incident illumination). However, the FACS provides a rapid objective and quantitative evaluation of cell surface binding reactions. In addition, fluorescent and non-fluorescent cells can be physically separated for further independent analysis.

Full experimental details of the development and application of these probes are published elsewhere (2, 4, 5, 16, 17). In this paper an up to date summary of results is provided.

Materials and Methods

Patients:

Leukaemia patients studied were attending clinics at St. Bartholomew's Hospital, London, the Hospital for Sick Children, London, or the Hammersmith Hospital, London. Diagnosis of their leukaemia was by standard clinical and haematological criteria (i.e. morphology and staining with Sudan Black and periodic acid Schiff). Blood and bone marrow samples, and in cases of central nervous system relapse, cerebro-spinal fluid, were taken. In some cases (particularly untreated AMLs) circulating white cells were removed using an IBM cell separator.

Preparation of cell suspensions:

In high count leukaemias the buffy coat was taken for study. In other cases heparinised blood or marrow were separated on ficoll-isopaque density gradient (18). Control, non-leukaemic cell suspensions were obtained from blood, tonsils, and bone marrow (ribs removed during thoracic surgery) and treated similarly.

Analysis and separation of fluorescent cells using the Fluorescence Activated Cell Sorter:

The Fluorescence Activated Cell Sorter (FACS)[1] is a recently developed and potentially extremely important automatic electronic device currently in operation in five or six laboratories, including our own (2). This machine has the dual capacity to rapidly and accurately analyse cells in suspension, in terms of size and fluorescence, and also to separate cells that are of particular interest. The principles involved in these two procedures are simple and have been described in detail previously (19, 20).

Figure 1 shows a simplified diagram which illustrates the general principles. The cells are contained in saline and emerge essentially in single file from an ultrasonically vibrated nozzle which eventually breaks up into regularly spaced droplets forming a stream of 50 μ diameter. The beam from an argon-ion laser intersects the cell stream just below the nozzle. As individual cells pass through the beam they scatter some of the light, and, if labelled, they also fluoresce. The two types of signals from cells are detected separately (using microscope objectives), amplified and converted into voltage pulses, the size of which is proportional to the input signal. After pulse height analysis these data are displayed in the form of a histogram; the ordinates representing the number of events (i.e. cells) recorded against pulse height (i. e. cell fluorescence intensity or size) – see following sections. Cells having particular characteristics of interest can be both enumerated and physically separated from the remainder. Separation is achieved by imparting a charge to the droplet stream. By 'informing' the FACS of the characteristics of the cells to be separated (e.g. fluorescent versus non-fluorescent, small cells versus large cells) and by finely controlled timing, droplets containing cells of interest charged and deflected into collection tubes, as they fall between charged plates (see Fig. 1). The time taken to accurately enumerate and separate various cell populations is determined by the flow rate. This will vary depending upon the particular experiment, but is

[1] Manufactured by Becton Dickinson Limited, Mountain View, California. Similar instruments are also currently under manufacture by other companies.

obviously geared to the rarity of the particular cells one is interested in. In general for analytical purposes, we screen lymphocyte preparations at 10^3–4 x 10^3 cells/second and red cell suspensions at up to 10^4 second, an analysis of 40,000 lymphocytes in the presence of red cells taking only a few seconds. For separation of relatively minor cell populations (see below) and for high purity, a lower sample flow rate of 1–3 x 10^3/second is usually employed. The FACS has the great advantage of being able to analyse very large numbers of cells at high speed and to efficiently separate reasonable numbers for functional analysis. Separations can be performed under sterile conditions with minimal loss of cell viability and function. There are no theoretical limits to the soluble fluorescent probes employed (e.g. lectins, antibodies, toxins, etc.). In general, cell surface reactive probes have been used; however, intracellular fluorescein diacetate can be used to distinguish live from dead cells (only the former give cytoplasmic fluorescence) and acriflavine and mithramycin dyes which bind to nucleic acids have been used to map cell cycle positions. As the sensors in the sorter measures only total energy, its function is independent of the cellular distribution of fluorescence (i.e. intracellular versus cell surface, random, diffuse versus concentrated or localised).

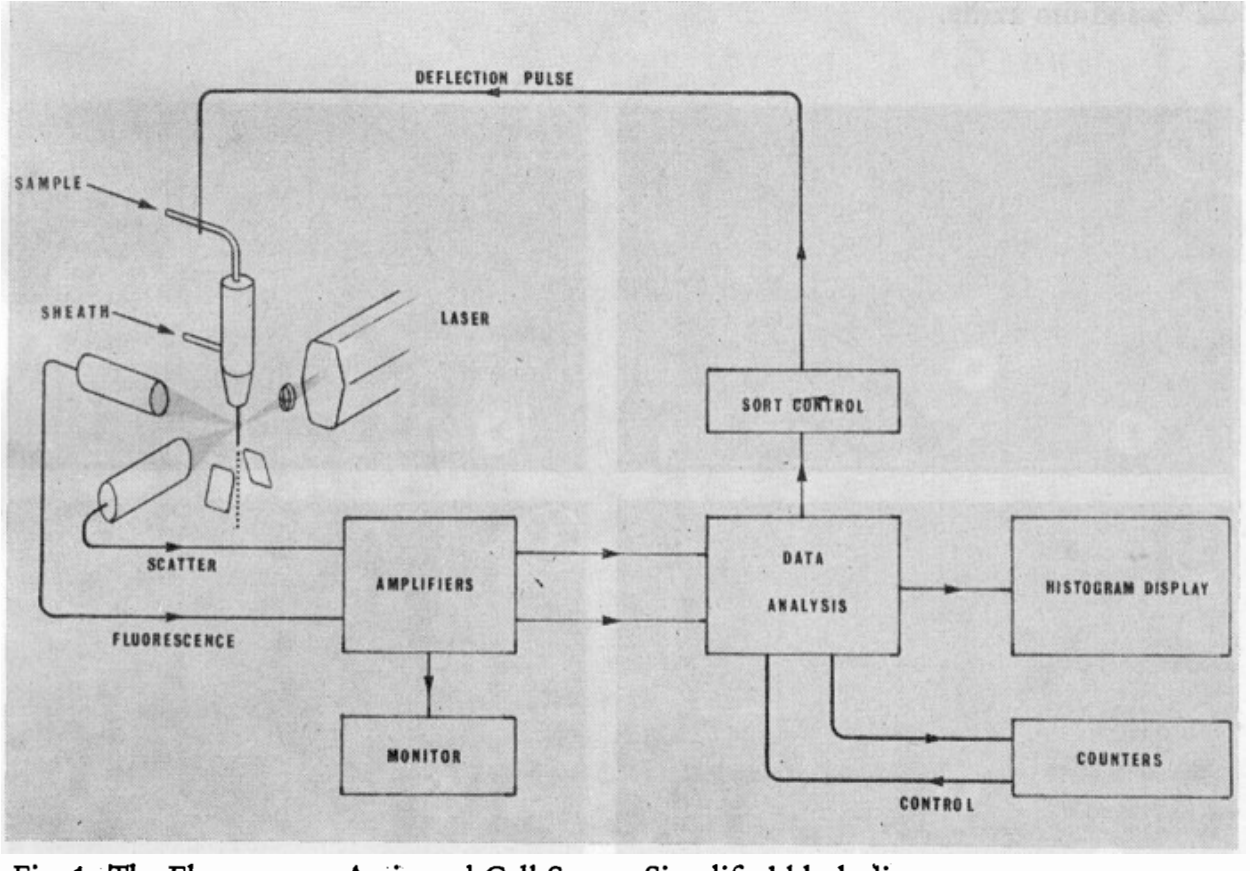

Fig. 1: The Fluorescence Activated Cell Sorter. Simplified block diagram.
Legend: See text for explanation.

Reagents:

Cholera toxin (choleragen) and horse anti-toxin were gifts from Dr A. Finkelstein. The former is now distributed through Schwartz-Mann Antisera to All was produced in rabbits as previously published (16). In brief, rabbits received two intravenous injections of viable ALL cells pre-coated in vitro with antibodies produced in rabbits against normal lymphocyte antigens. Following heat inactivation of complement components the sera were absorbed with red cells, liver homogenate, tonsil lymphocytes, AML cells and normal bone marrow cells. Absorptions were checked for efficiency and completeness using the Fluorescence Activated Cell Sorter (17).

Immunofluorescent labelling of cells:

Cells were labelled on their surfaces by indirect immunofluorescent methods. Binding of cholera toxin was detected by sequential treatment of cells with appropriate concentrations of cholera toxin, horse anti-toxin, followed by fluoresceinated rabbit anti-horse IgG. Binding of anti-ALL was similarly detected by adding sequentially rabbit anti-ALL followed by fluoresceinated goat anti-rabbit IgG. In standard tests 10^6 cells were used (in 100 µl). The first ligand was added at room temperature and subsequent steps performed at 4 °C in the presence of 0.2 % sodium azide.

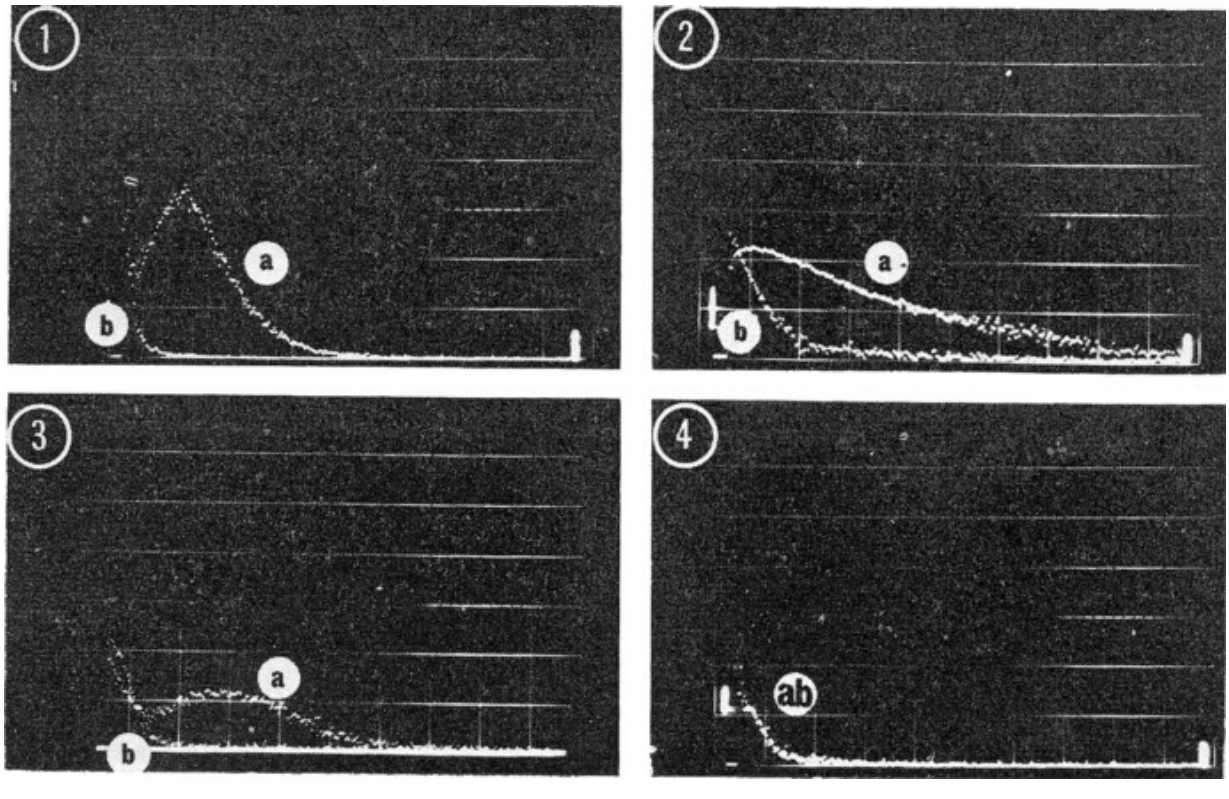

Fig. 2: Fluorescence Activated Cell Sorter (FACS) analysis of cholera toxin binding.
Legend: Vertical axis: relative cell number. Horizontal axis: relative fluorescence intensity.
1. Thymus cells (4 yr donor, cardiac surgery) (a) cholera toxin, (b) control (see methods).
2. Tonsil lymphocytes (a) cholera toxin (b) cholera toxin pre-incubated with G_{M1}.
3. Chronic lymphocytic leukaemia cells (a) cholera toxin, (b) control.
4. Acute lymphoblastic leukaemia cells (a) cholera toxin, (b) superimposed control.
Taken from ref. 21.

Results and Discussion

1. Binding sites for cholera toxin

A variety of human leukaemias have been analysed for cell surface cholera toxin binding sites (21). Results to date (Autumn, 1975) are given in Table 1 and Fig. 2. Irrespective of morphological type all chronic leukaemias have cholera toxin receptors whereas acute leukaemias have few if any. Philadelphia chromosome positive CML, like CLL, has an apparently normal expression of G_{M_1}. CML usually progresses to a terminal phase characterised by blast crisis relapse. This is generally regarded as an acute transformation of the Ph^1 positive malignant clone (22). As indicated in Table 1 blast crisis relapse cases of CML in contrast to CML itself lacked cholera toxin receptors. This observation re-inforces the argument that this test discriminates between acute and chronic phase cells. The exact proportion of G_{M_1} negative cells in blast crisis relapse was quite variable and each patient had a mixed population of blast cells and granulocytic cells. This point can be clearly illustrated by cell sorting experiments. The FACS was used to identify and separate

Table I: Cholera toxin staining of human leukaemia cells

Leukemia type	Cholera toxin labelling		
	+	±	–
ALL – untreated (or relapse)			
1. Non-T, non-B*			12
2. T**			3
3. B (Burkitt-like)***			2
ALL – in remission	2		
AML – untreated	1	4	9
AML – in remission	3		
CLL	11		
CML (Ph^1+)	16		
CML in blast crisis (Ph^1+)			20
Others:			
Sezary syndrome		1	
Prolymphocyte leukaemia	3	1	2
Hairy cell leukaemia			2

\+ 75–100 % cells strongly positive.
± 15–75 % of cells weakly positive.
– < 15 % of cells weakly positive.
* E rosette: neg, SmIg: neg, anti-ALL: pos.
** E rosette: pos, SmIg: neg, anti-ALL: neg.
*** E rosette: neg, SmIg: pos, anti-ALL: neg.
Taken from ref. 21.

$G_{M1}+$ and $G_{M1}-$ cells in a case of blast crisis of CML (Fig. 3). The separated fractions were re-run in the FACS for analysis and also smeared onto slides and stained. The results showed that G_{M1} negative cells were exclusively undifferentiated blast cells whereas the positive cells were granulocytic. These data suggest that acute blastic transformation in CML might be detected at an early stage by the appearance of G_{M1} negative cells. Negative acute leukaemias can be converted into positive cholera toxin binders by two simple manoeuvres (Fig. 4): (i) insertion of purified G_{M1} ganglioside (the receptor for cholera toxin – ref. 12) into the cell membrane of the leukaemic cells. (ii) Treatment of cells with neuraminidase which cleaves off sialic acid residues from more complex gangliosides converting them into monosialoganglioside G_{M1} in which the single sialic acid residue is sialidase resistant.

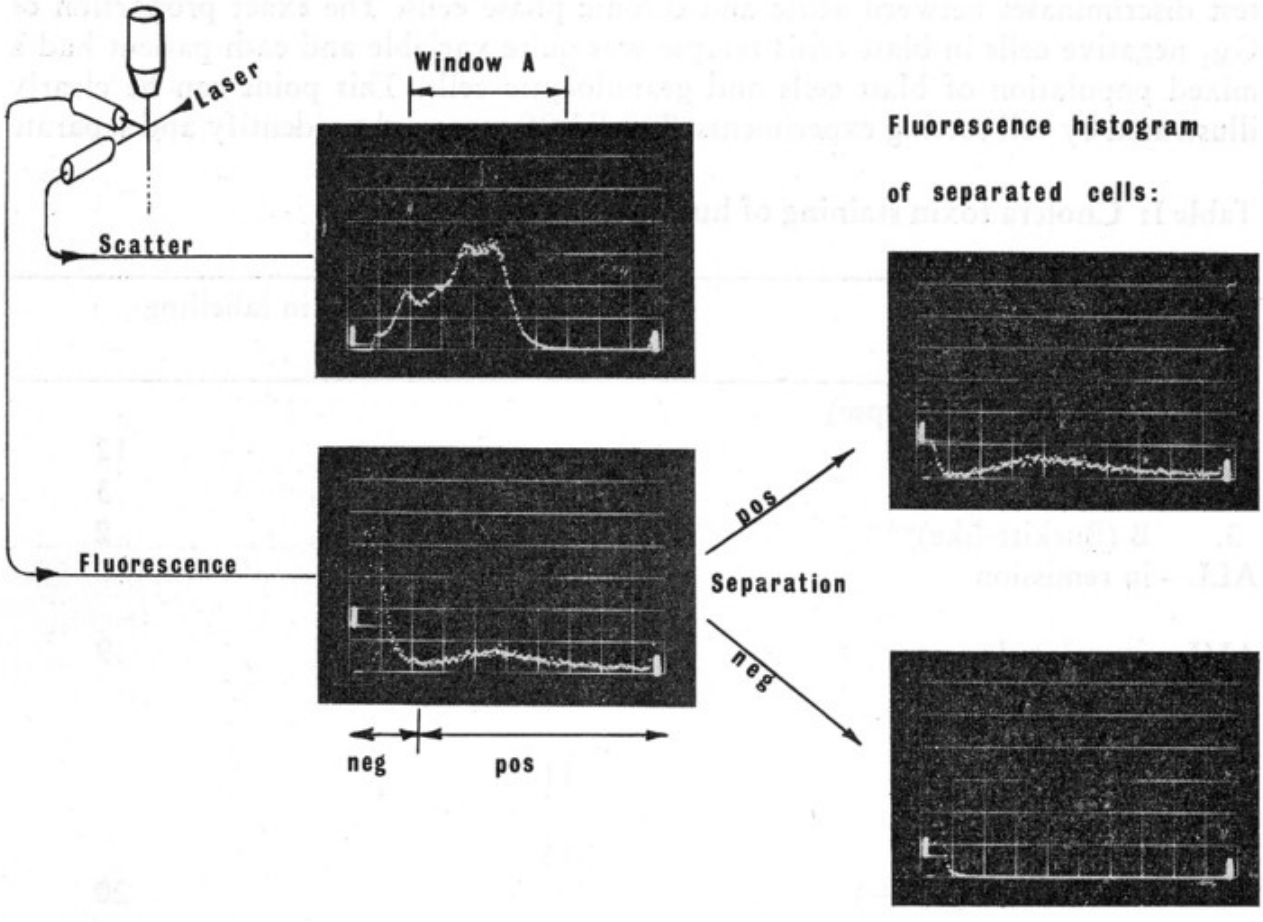

Fig. 3: Separation of positive and negative cholera toxin binding cells from a blood sample of a CML patient in blast crisis relapse.
Legend: see Methods section for technique taken from ref. 23.

We take these observations to imply that there is no defect at the level of G_{M1} insertion in the membrane and that the ganglioside deficiency in acute leukaemia may be more pronounced in G_{M1} than other more complex gangliosides. The latter observation contrasts with what is seen when the same experiments are carried out with the transformed BHK (hamster kidney) cell line which has been shown by chemical criteria to be defective in all gangliosides (24 and D. Critchley, personal communication). Here neuraminidase treatment is without effect (Fig. 4).

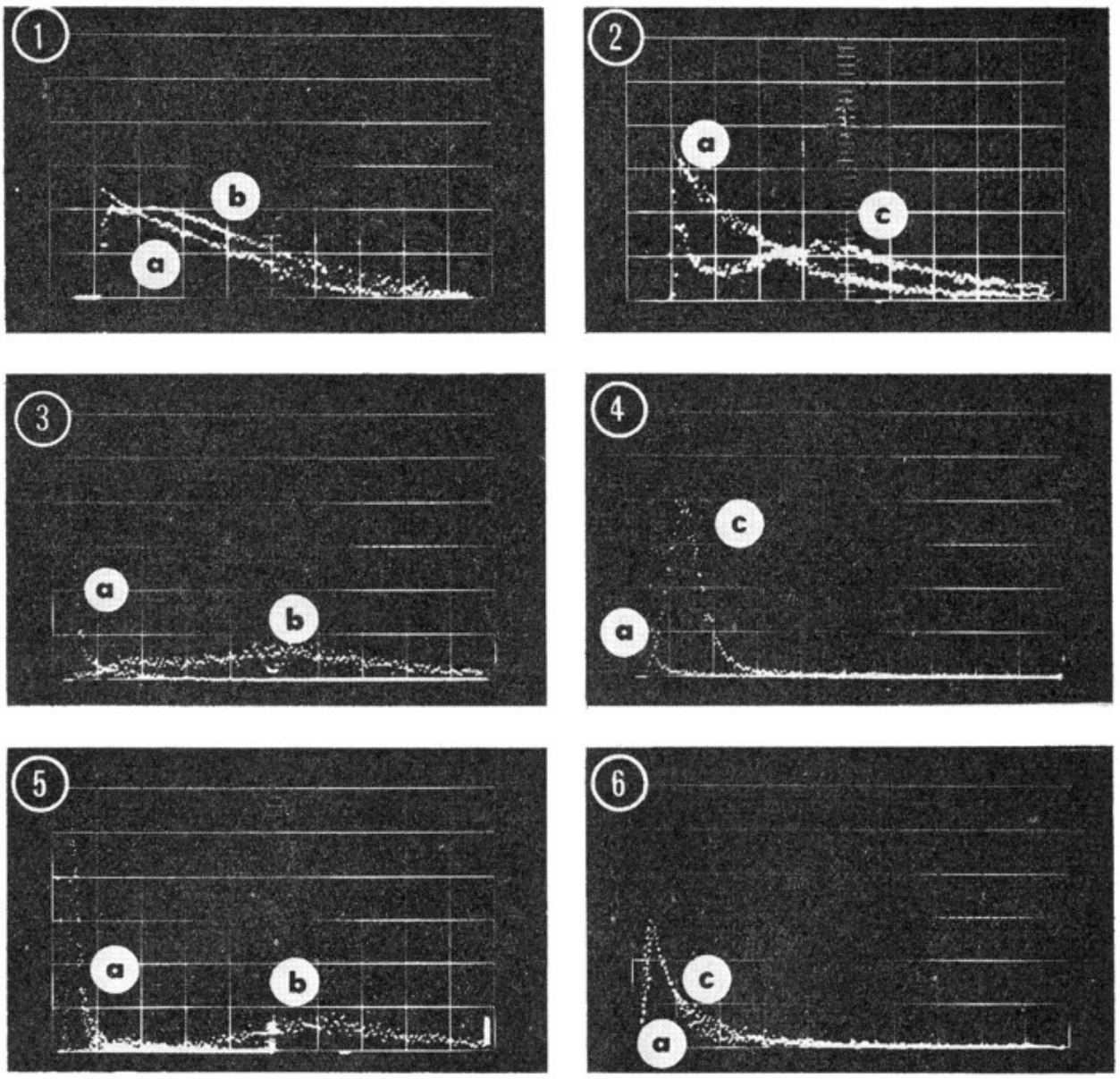

Fig. 4: Reconstitution of G_{M1} receptors for cholera toxin.
Legend: FACS analysis, axes as in Fig. 2.
(1) and (2) tonsil cells, (3) and (4) acute leukaemic cells, (5) and (6) Transformed Hamster BHK fibroblasts.
(a) cholera toxin binding, (b) cholera toxin binding after G_{M1} insertion into cells (see methods), (c) cholera toxin binding after treating cells with neuraminidase.
Taken from Ref. 21.

Although the G_{M1} deficit in acute leukaemia may be fairly selective, preliminary chemical analyses of these same cells suggest that a general reduction of charged glycolipids may exist (B. Murray and M. Greaves, unpublished observations). These observations are consistent with the view that a simplification of glycolipids occurs in transformed cells. The primary locus for the effect may be at the level of glycosyl transferase enzyme activity (13, 25).

Precisely what a deficiency in cholera toxin receptors can tell us about the acute leukaemia cell is unclear. Three general interpretations can be considered: (i) the deficiency is a primary or indirect consequence of the malignant process itself; (ii) the variable expression of cholera toxin binding sites (probably G_{M1} molecules) in

leukaemia reflects cell cycle position or general growth/proliferative status independent of the neoplastic condition; (iii) the expression of cholera toxin binding sites on leukaemic cells simply reflects the status of these same structures on the normal cellular counterpart or progenitor, i.e. acute leukaemias are a malignant derivative of a (undifferentiated?) cell which itself has no receptor for the toxin. We cannot as yet determine which of these three, if any, is correct. However, several clues are available. A number of normal proliferating and non-proliferating cell types have also been tested for cholera toxin receptors and the only negative cells so far identified are members of the erythroid series (Table 2). Significantly, EBV transformed lymphoid (B) cell lines are positive although possibly less so than non-dividing B lymphocytes. Thymocytes and T lymphocytes are also positive which implies that T-ALLs may differ from their normal cellular counterparts or progenitors. The normal function of G_{M1} is inknown. Indirect evidence suggests this and other glycolipids might play an important role in growth control (13) but this remains to be clearly established. G_{M1} negative human and murine leukaemias and lymphomas are available (De Cicco and Greaves, unpublished observation), and the easy insertion of G_{M1} into such cells suggests that it may be possible to determine the influence of G_{M1} molecules on cell growth and malignancy.

Table II: Cholera toxin staining of normal human cells

Cell type	Cholera toxin labelling	
	% Positive	Intensity of staining
Red blood cells	0	–
Polymorphs	98	+++
Monocytes	95	+++
Peripheral lymphocytes	92	++
Thymocytes	99	++
Spleen cells	96	++
Tonsil cells	95	++
Bone marrow	85	++→+++
Cord blood		
Polymorphs	99	+++
Lymphocytes	92	++
Normoblasts	0	–
PHA lymphoblasts	95	++
B-lymphoblasts	78–95	+/++
B-lymphoid cell lines (11)		

Taken from ref. 21.

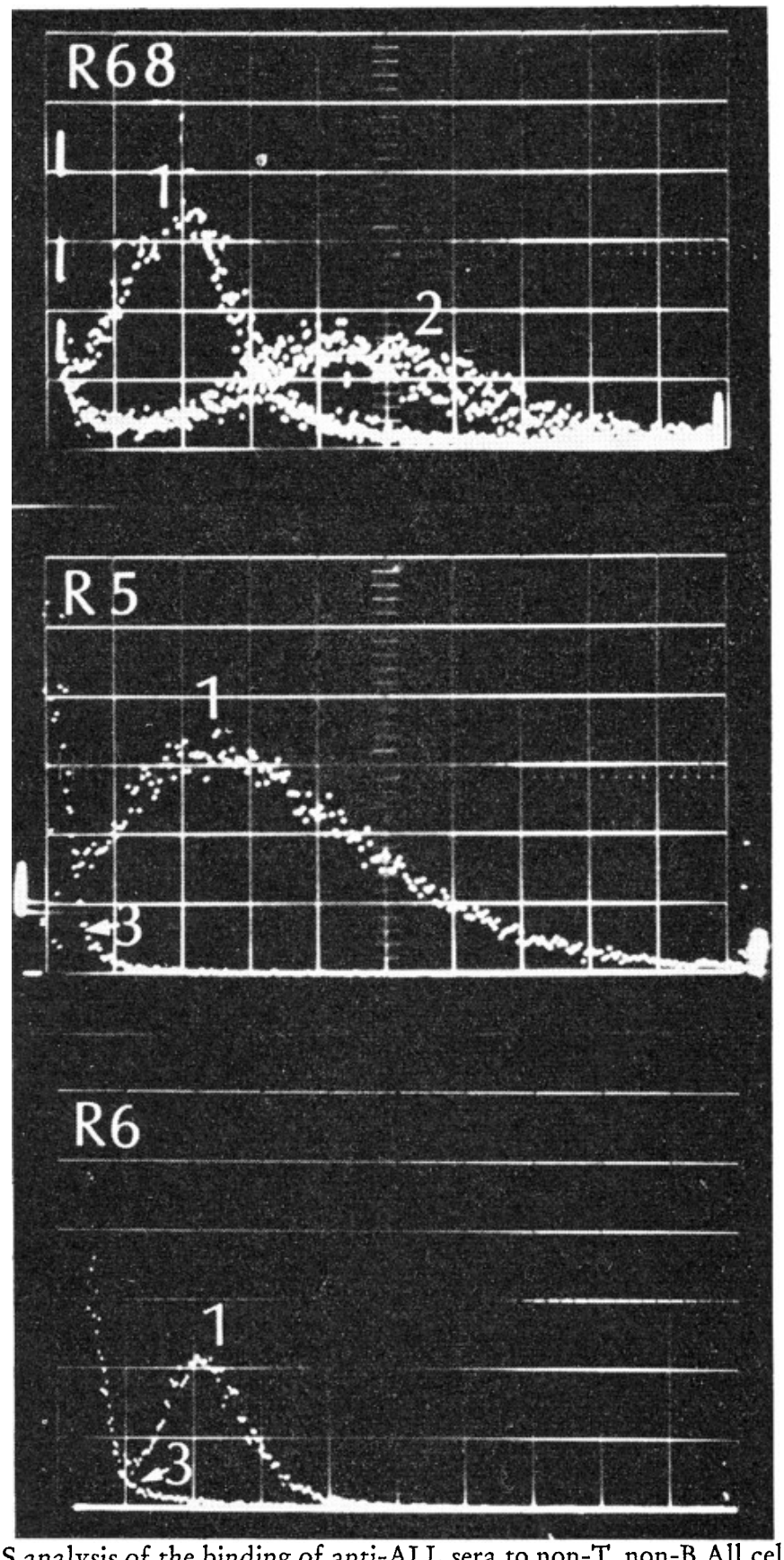

Fig. 5: FACS analysis of the binding of anti-ALL sera to non-T, non-B All cells.
Legend: Ordinate: relative cell number.
Abscissa: relative fluorescence intensity.
R68, R.5 and R.6 – three different rabbit sera.
1. Fully absorbed sera (red cells, liver, lymphocytes, bone marrow, AML).
2. Absorbed red cells, liver, lymphocytes only.
3. Controls: normal rabbit serum or anti-ALL absorbed with ALL cells.
Taken from ref. 17.

2. Antisera to common (non-T, non-B) ALL

After absorption with red cells, liver, lyphocytes, AML and bone marrow, antisera to ALL are specific for ALL as judged by indirect immunofluorescence and absorption tests (16, 17). Table 3 lists non-leukaemic cells which have been shown to be negative. The antigen(s) being detected would appear *not* to be a cryptic, cell cycle or foetal (phase specific) determinant.

Table III: Possible antigenic specificities defined by antisera to ALL

Potential Antigen	Cells tested	Cross reactive with anti-ALL?[1]
1. 'Normal' antigen	thymus*, tonsil*, appendix, blood w. b. c., spleen, bone marrow*, liver*	No[2]
2. 'Cryptic' normal lymphocyte antigen	Tonsil lymphocytes treated with trypsin[3], pronase[4] or neurominidase[5]	No
3. Lymphocyte antigens expressed during mitosis	Mitogen activated tonsil T and B lymphocytes[6]. Lymphoblastoid cell lines[7]. Lymphoblasts from blood of patients with infectious mononucleosis	No
4. 'Foetal' antigens	Spleen 13–22 wk, Thymus 15–22 wk, liver (9–22 wk)*	No

[1] All cells tested by indirect immunofluorescence with anti-ALL. Cell types marked with asterisk * were also tested for their capacity to absorb out anti-ALL antibodies.
[2] No reactivity = less than 0.5 % positive cells recorded which is the "background" recorded with normal control sera, or alternatively, in absorption experiments, reflects no shift in the FACS profile on anti-ALL against ALL cells.
[3] Trypsin (Sigma 12,000 BAEE units/mg. 2X crystallised) 50 μg/10^6 cells/1 ml/30 min/37 °C.
[4] Pronase (BDH 45,000 PuK units per g) 50 μg/10^6 cells/1 ml/30 min/37 °C.
[5] Neurominidase (vibrio cholera Calbiochem. Grade B) 0.5 u/10^6 cells/1 ml/30 min/37 °C.
[6] Tonsil T lymphocytes cultured with phytohaemagglutinin or Concanavalin A or spleen B lymphocytes cultured with pokeweed mitogen.
[7] Derived from normal adult blood or from blood of patients with infectious mononucleosis, Burkitt's lymphoma or ALL.

Figures 5 and 6 illustrate reactivity of leukaemic cells with anti-ALL as analysed by the FACS. In Table 4 the pattern of reactivity in leukaemias is presented. Virtually every common ALL reacts whereas all T and B ALLs do not. Other types of leukaemias do not react, with two interesting exceptions – AUL and CML in blast crisis. Six out of eight AUL patients and nine out of 19 patients with CML blast crisis had a high proportion of cells reacting with the anti-ALL serum. Blast cells in CML acute crisis can be predominantly 'lymphoid' or 'myeloid' by morphological and cytochemical criteria (22). ALL antigen positive cells were only found in the 'lymphoid' crisis, although not in all such cases. In all

cases studied, however, the blast crisis involved Ph[1] positive cells (23). In one particularly interesting case an ALL antigen negative 'lymphoid' blast crisis in blood was followed by CNS relapse which was ALL positive (i.e. the cells in cerebro-spinal fluid). In the same sample, all metaphases were Ph[1] positive. These results provide direct evidence for earlier suggestions that CML can enter an ALL-like phase (26, 27) and parallels recent findings using terminal deoxynucleotidyl transferase enzyme as a marker for ALL (28, see also Sabin and McCaffrey in this symposium).

Table IV: Reactivity of different leukaemias with anti-ALL serum[1]

Diagnosis:	ALL								
	Common, non-T non-B, ALL	T-type ALL	B-type ALL	AUL	AML	AMML	CML	CML-BC[3]	CLL[4]
Proportion of positive cases:	47/50[2]	0/14	0/3	7/9	1/48	0/10	0/10	9/19	0/12

[1] The sera were raised against lymphoblasts from children with typical non-T, non-B type ALL.
[2] Pooled data from adults and children.
[3] Chronic Myeloid Leukaemia in Blast crisis relapse (see ref. 23). All nine positive cases had a 'lymphoid' morphology. All 19 cases were Ph[1] chromosome positive.
[4] All leukaemias were diagnosed (using marrow or blood cells) by standard morphological and histochemical methods (including Romanowsky, Sudan Black, periodic-acid Schiff and Acid phosphatase staining).

The most likely interpretations of these findings are that: 1. ALL of both children and adults can involve three different cell types and therefore probably derives from three different progenitors – T lymphocyte related cells (probably thymocytes), B lymphocyte related cells, and in the majority of cases, a non-T, non-B cell which could be either a lymphoid precursor (28) and/or a pluripotential stem cell. 2. Common ALL, most AUL cases and a proportion of CML blast crises involve the same cell type. Important corollaries of this are that (i) CML is, in at least some cases, a malignancy induced in a pluripotential stem cell rather than a myeloid stem cell. A similar suggestion was made previously based on the results referred to above with terminal transferase enzyme (29). (ii) the ALL antigen(s) can be expressed in diseases involving a common target cell but a different aetiology (e.g. Ph[1] positive or negative). This might lead one to suspect that the ALL antigen would be found on the normal counterpart or progenitor of the 'ALL' spectrum of diseases. If this is the case then we can say that this cell has a frequency in formal bone marrow of less than 0.1 %. This in turn implies either a derivation of ALL from a very rare cell (e.g. a pluripotential stem cell) or that the ALL antigen(s) is only expressed on transformed derivatives.

The nature of ALL antigen has recently been investigated (30). It is glycoprotein as judged by sensitivity to pronase and interaction with lentil lectin. It does not

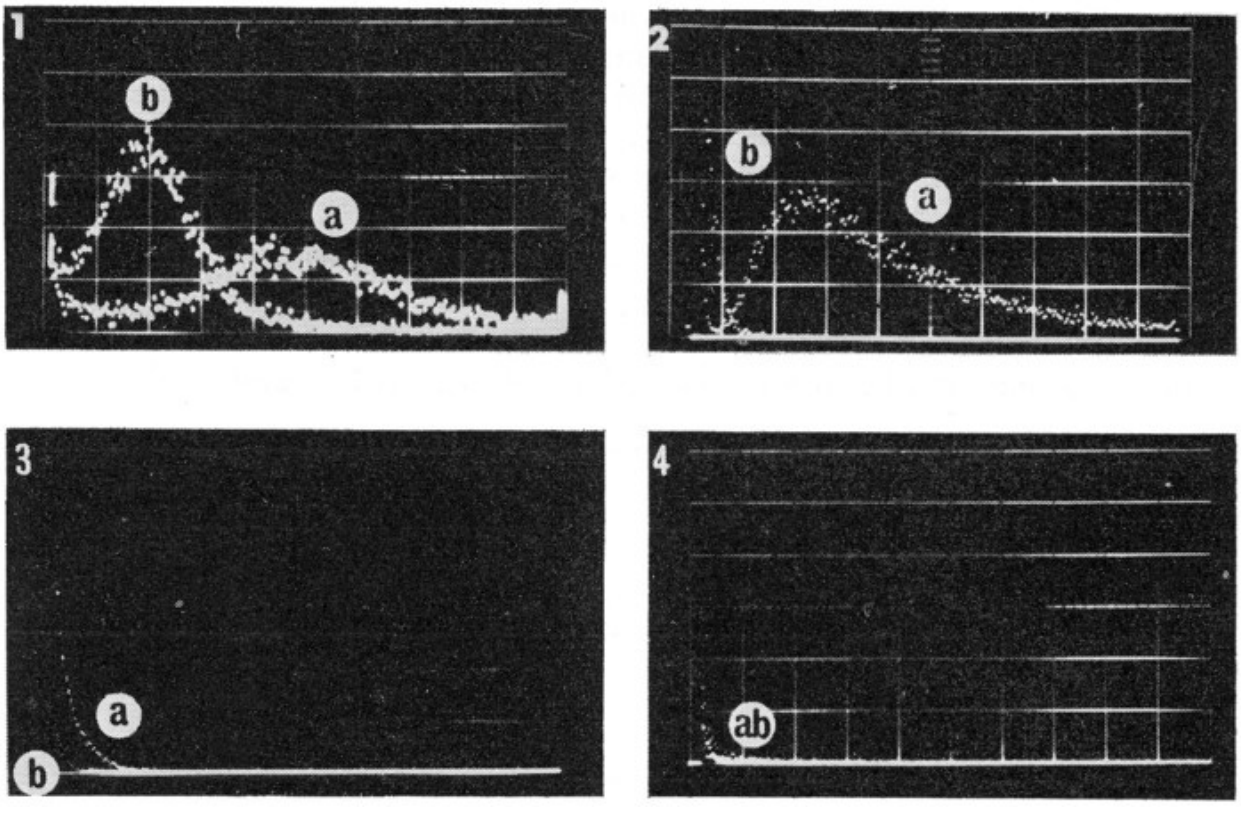

Fig. 6: FACS analysis of the binding of anti-ALL sera to different leukaemias.
Legend: Ordinate: relative cell number.
Abscissa: relative fluorescence intensity.
1. Untreated non-T, non-B ALL (Blood) as in Fig. 5.
2. Non-T, non-B ALL (CNS relapse) cerebro-spinal fluid.
3. Untreated AML (Blood, IBM separated).
4. Untreated T-like ALL (Blood).
(a) Anti-ALL, (b) Control (as in Fig. 5).
Note small difference between (a) and (b) in 3 can be removed by a further single absorption with AML, cells (17).

appear to cross-react immunologically with a variety of C-type RNA oncornaviruses including murine Moloney and Gross viruses, Feline leukaemia virus, Simian Sarcoma virus and the recently isolated human leukaemia (AML) virus, HuLV-23-1 (31).

Selective binding of anti-ALL sera can be demonstrated in a quantitative manner by the iodinated anti-globulin binding method (Sutherland and Greaves, unpublished observations). This result also raises the possibility of developing a sentitive radioimmune assay for cell-free leukaemic antigens in patients' serum and cerebro-spinal fluid.

The precise selectivity and sensitivity of the anti-ALL probe suggests that it should be possible to use it to detect rare leukaemia cells in situations where they would appear to be absent by standard haematological tests. We have been able to do this in two situations so far. A proportion of untreated ALL patients present with a typical lymphoblastic bone marrow but with no obvious leukaemic cells in the peripheral blood picture. Five of such patients (included in Table 4) we studied, had no leukaemic blasts by routine haematological assessment, but we

found 1 to 5 % leukaemic cells in the blood of all five, using anti-ALL sera. This result suggests that anti-ALL antibodies could be used to detect early emergence of the leukaemic clone into the circulation. One would obviously like to detect early expansion of the malignant cell in the marrow itself. One approach to this problem is to study the marrow of patients prospectively through treatment, in order to assay for residual disease and/or early signs of relapse. Such trials are in progress and, meanwhile, spot checks on individual patients indicate that rare leukaemic cells may indeed be present in the bone marrow of patients considered to be in complete remission (17).

The availability of antisera to leukaemia specific or leukaemia associated antigens raises the possibility of therapeutic applications. Our antisera are not directly cytotoxic (in the presence of complement) and we are currently exploring their potential use as selective 'bullets' in antibody dependent cell induced killing systems (cf. ref. 32) and as carriers of cytotoxic drugs (cf. ref. 33). It is also possible that the leukaemic antigen(s) itself, once isolated, could be rendered highly immunogenic and re-introduced to the patient.

In conclusion, we suggest that fluorescent cell surface binding probes provide a new incisive tool for characterising or 'phenotyping' leukaemic cells. Table 5 is a summary of all the membrane markers we have studied and the current distribution of reactivity among different leukaemias. Of these various test systems anti-leukaemia antibodies in combination with analytical efficiency of the Fluorescence Activated Cell Sorter provide a particularly exciting approach for accurate

Table V: Cell surface phenotype of human leukaemic cells tested at University College (1972–1975)

Membrane markers	Refs.	ALL			AUL	AML	AMML	A. Mon. L	CML	CML-BC	CLL
		Common or non-T, non, B type	T-type	B-type							
A. LYMPHOCYTE MARKERS											
i. Anti-lymphocyte serum[1]		▨	▨	▨	▨						▨
i.i. T-markers. Anti-T serum[2] E rosettes	(4, 2)		▨								
i.i.i. B-markers. Anti-Ig serum Anti-CLL (B) serum	(4, 2, 34)			▨							▨
B. MYELOID MARKERS											
i. Anti-monocyte serum	(5, 2)						▨	▨			
i.i. Anti-granulocyte serum[3]	-					▨			▨	▨	
C. LEUKAEMIA MARKER Anti-ALL serum	(16, 17, 30)	▨			▨					▨	
D. CHOLERA TOXIN	(21, 2)								▨		▨

[1] Rabbit anti-thymus serum absorbed with normal blood granulocytes and AML cells (Brown & Greaves unpublished observations).
[2] Antisera to thymus or brain (4, 2).
[3] Kindly provided by Dr. A. B. de la Riviere. This rabbit serum was raised as previously described by Engelfriet et al. (35).
Shaded areas represent proportion of positive cases.

and sensitive diagnosis of disease and monitoring the response of patients to therapy.

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