Leukemic Cell Phenotypes in Man: Relationship to 'Target' Cells for Leukemogenesis and Differentiation Linked Gene Expression

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Established cell lines derived from malignant cells are proving to be of considerable value in the investigation of differentiation and controls of gene expression. This is especially evident in the leukemias; the Friend virus-induced erythroleukemia (Harrison, 1977), avian erythroleukemia (see Graf, this book) and myeloid leukemias in rats (Lotem and Sachs, 1974) and a recently described human myeloid line (Collins et al., 1978), all having a maturation arrest which can be reversed by various agents and to some extent controlled by viruses (see papers by Gallo, Ruscetti and Graf in this book).

During the past few years we have developed reagents for an immunological analysis of acute and chronic leukemias in man and permanent lines established from these cells (reviewed in Greaves, 1975; Greaves and Janossy, 1978; Thierfelder et al., 1977). Part of the incentive has been to provide a routine immunodiagnostic service; this is now operational and effectively identifies subgroups of Acute Lymphoblastic Leukemias (ALL) which are distinct in terms of prognosis (Chessells et al., 1977; Greaves, 1978; see also Pinkel, D., this book). A longer-term interest has been to use ALL cells and their cell line derivatives for analysis of early lymphoid cell development and diversification. Table 1 lists the antisera and enzyme assays that we use to characterize leukemic cells. All the antisera are directed against cell surface determinants and their binding is detected by immunofluorescence and with the Fluorescence Activated Cell Sorter (FACS) as previously discussed (Greaves, 1975; Greaves et al., 1976).

1. Subgroups of Acute Lymphoblastic Leukemia and Lymphoid Lineage Differentiation

Fig. 1 illustrates the heterogeneity of ALL as revealed by the various immunological markers and enzyme assays. Four major groups are identified and within these fairly clear subsets. The four subgroups have different prognosis in both children (Chessells et al., 1977) and adults (Lister et al., 1979). These profiles are assembled from the analysis of over 1000 acute leukemias and we believe that they reflect relative stable maintenance of normal gene expression by the major subclones in these leukemic patients, i.e. the phenotype of each type is appropriate for the developmental level at which maturation arrest has occurred and the markers are in essence differentiation-, or matura-

Table 1. Markers used to Phenotype Human Leukemic Cells[a]

[a] Details of these markers are reviewed in Greaves (1975) and Greaves and Janossy (1978). References for individual markers are also given in the text of this paper

Membrane markers	
Antisera:	Anti-ALL
	Anti-p28,33 ('Ia')
	Anti-HuTLA (T cell antigen)
	Anti-M (Myelomonocytic antigen)
	Anti-L (Lymphoid antigen)
	Anti-SmIg (membrane immunoglobulin)
Sheep erythrocyte rosettes	
Mouse erythrocyte rosettes	
Cholera toxin	
Enzyme markers	
Terminal deoxynucleotidyl transferase (TdT)	
Hexosaminidase isoenzyme (HEX-I)	
Acid phosphatase (AP)	

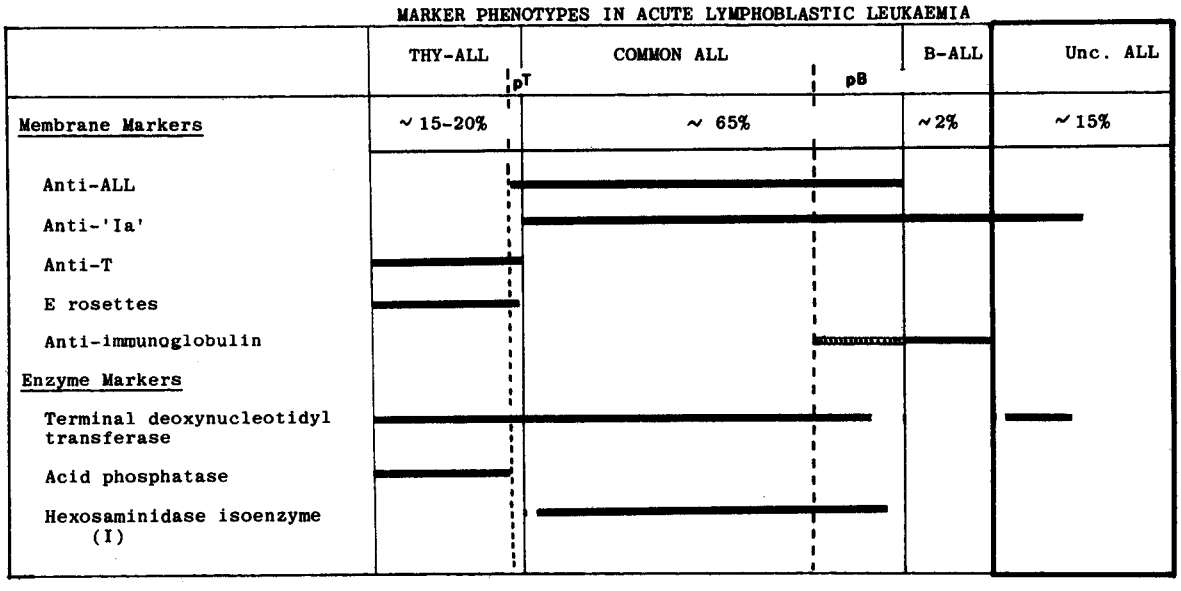

Fig. 1. Major phenotypes observed in Acute Lymphoblastic Leukemia (ALL). The solid horizontal bars are proportional to the number of patients positive for the given marker. Over 1000 patients (~90% untreated. 10% in relapse) have been tested with anti-ALL. anti-SmIg and E rosettes and between 100 and 500 tested with other markers. Results from children (80% of cases) and adults have been pooled to construct the above profile; the size of subgroups is, however, different in children and adults. In children 75% of ALL's are anti-ALL serum positive and 15% of T-ALL's whereas in adults (> 16 years) only 50% are anti-ALL positive and 10% are T-ALL's. Thus, the unclassified category is larger in adults.
Unc: Unclassified ALL ($cALL^-$, T^-, E^-, $SmIg^-$)

tion-linked. As discussed elsewhere (Greaves and Janossy, 1978) there is no evidence that any of these antisera detect leukemia-specific antigens; furthermore we have questioned whether any such antigens exist with any consistency (Greaves and Janossy, 1978).

It seems plausible that the subgroups of ALL identified represent virtually all the early lymphoid lineage maturation compartments, the subgroups being overlapping rather than completely distinct. A 'polarity' of the groups can be revealed by the overlap of individual characteristics. Thus a subset of T-ALL ($\sim 10\%$) have only a partial T or thymic phenotype (see Fig. 1) and express the cALL antigen, albeit weakly. We refer to these as 'early' or pre-T's. Similarly within the major or common ALL subgroup are leukemic cells with 'early' or pre-B features, i.e. cytoplasmic IgM but no detectable cell surface Ig (Vogler et al., 1978; Greaves, Verbi, Vogler and Cooper, unpublished observations). The 'unclassified' or 'null' subgroup in turn has phenotypic overlap with the cALL group by expressing (in some cases but not all) TdT and Ia antigens. We suspect therefore that these profiles may reflect fairly accurately normal patterns and sequences of gene expression in lymphocyte ontogeny. To establish this hypothesis experimentally requires identification of normal precursors with equivalent phenotypes and an in vitro system in which sequential maturation linked phenotypes can be induced (see below).

ALL is principally a proliferation of immature lymphoid cells. Several types of T and B neoplasms exist, however, where the majority of cells are 'frozen' at levels equivalent to relatively mature lymphocyte subsets. This point has been emphasized by Salmon and Seligmann (1974) in the context of B cell leukemias (CLL), lymphomas and myeloma and seems also likely to be correct for T lineage malignancy. In Fig. 2 we have constructed a tentative cell lineage map in which the leukemic phenotypes we have observed are placed in position and sequence according to a possible lineage order of gene expression.

2. Molecular Nature and Cellular Selectivity of Expression of the cALL Antigen

In hemopoietic malignancy the cALL antigen is detected only in those which are considered lymphoid or undifferentiated, i.e. AUL, cALL and Ph^1 positive 'lymphoid' blast crisis of CML (Greaves et al., 1977; Roberts et al., 1978). Cross-absorption studies indicate that the same antigenic determinant is found in these different lymphoid leukemias (Roberts et al., 1978); in addition the antigen has the same general molecular characteristics when isolated from Ph^1 positive 'lymphoid' blast crisis, cALL and T-ALL cell lines (Sutherland et al., 1978). The cALL antigenic determinant is found on a single glycosylated polypeptide with an apparent molecular weight of 100,000 daltons in SDS polyacrylamide gel electrophoresis. This molecule is released or secreted by leukemic cells and leukemic cell lines and runs in the same position with and without reducing agent. All of the molecules bind to ricin lectin (which 'recognises' terminal galactose residues) but only

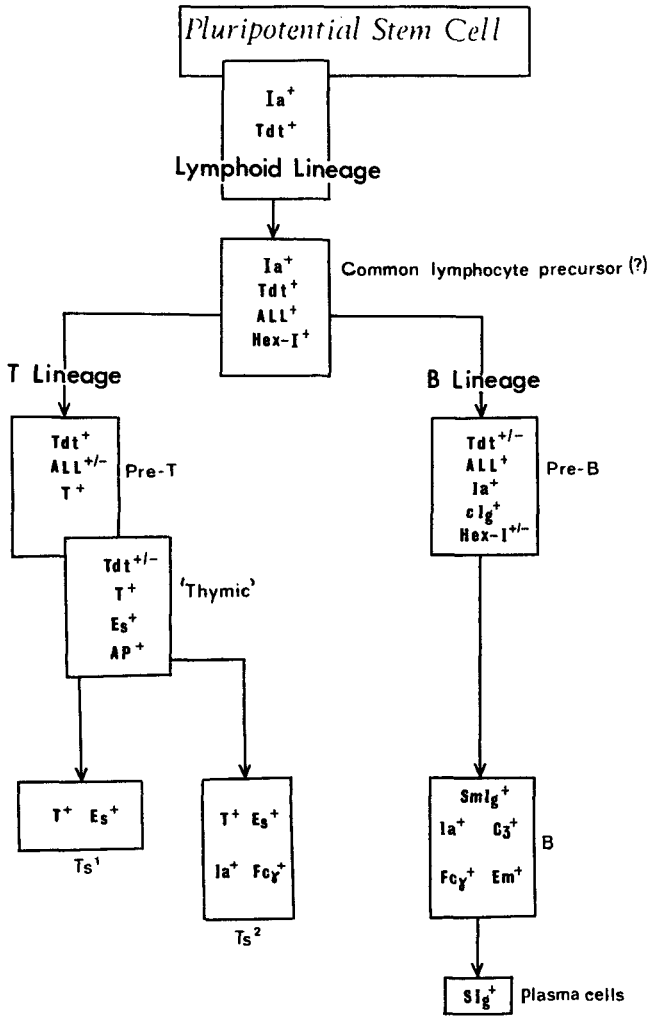

Fig. 2. A hypothetical map showing the sequence of gene expression in the lymphoid lineages as suggested by analyses of phenotypes of leukemic cells
+ positive8 – negative
See Table 1 for abbreviations.

Fcγ	Receptor for IgG
C3	Receptor for complement component C3
Em	Receptor for mouse erythrocytes
CIg	Cytoplasmic Immunoglobulin
SmIg	Surface membrane Immunoglobulin
SIg	Secreted Immunoglobulin

a fraction to lentil lectin (-terminal glucoside or mannoside residues); its isoelectric point is 5.6 (Smart et al. 1979).

Recently we have been able to identify a normal 'lymphoid' cell with ALL^+, Ia^+, T^-, $SmIg^-$ phenotype of cALL cells (Greaves and Janossy, 1978). We had previously failed to find such cells in normal adult marrow but now find them in fetal hemopoietic tissue, regenerating marrow (e.g. post-transplantation for aplastic anemia or post-chemotherapy for malignancy) and in neonatal, lymphoproliferative non-malignant 'leukemoid' reactions. Although the ALL antigen has not been isolated from these sources and shown to be identical with the antigen on leukemic blasts we conclude that the cALL antigen is most probably a normal gene product of lymphocyte precursors.

3. Acute Myeloid Leukemia (AML) Associated Membrane Antigens and Myeloid Lineage Differentiation

90% of the AML cases we have tested (~100) have been positive for the M (myeloid) antigen and negative for the ALL antigen (M^+, ALL^-) as are cases of CML (10/10) and a proportion of CML in blast crisis (17/25) (Roberts et al., 1978; Roberts and Greaves, 1978). In the majority (~90%) of cases of AML (Schlossmann et al., 1976; Janossy et al., 1977a) and Ph^1 positive CML

in blast crisis (Janossy et al., 1977a and b) the myeloblasts are also Ia or p28,33 positive; the granulocytic cells in CML itself are Ia negative (Janossy et al., 1977a and b). In our series 10/10 AML were Hex-I negative (Ellis et al., 1978) and 17/17 TdT negative ($\sim$0·2 μ/10^8 cells, Hoffbrand et al., 1977). The major phenotype of AML is therefore: M^+, Ia^+, ALL^-, Hex-I^-, TdT^- and, in addition, $HuTLA^-$, E^-, AP^+ (see in Tables 1 and 2). Myeloblasts in AML may sometimes be SmIg positive (k plus λ) due to adsorption of IgG to Fcγ receptors. There is often considerable variation in the levels of M and Ia antigens on cells from individual patients with AML or CML in blast crisis. This heterogeneity can be evaluated by the Fluorescence Activated Cell Sorter and has been shown to correlate with the degree of morphological maturation within the granulocyte lineage (Roberts and Greaves, 1978; Janossy et al., 1977a and b). Parallel studies on normal marrow and blood samples indicate an essentially similar relationship (Roberts et al., 1978; Janossy et al., 1977b; Winchester et al., 1977) suggesting that membrane marker expression on leukemia clones is maturation-linked and essentially normal. In our laboratory absorption of three rabbit and one rhesus monkey anti-Acute Myeloid or Myelomonocytic sera with normal bone marrow (M. Greaves, unpublished observations) removed all activity against leukemic cells indicating that these particular sera contained no leukemia specific antibodies. Mohanakumar and colleagues (Mohanakumar et al., 1975; 1976) have reported different results with various primate antisera.

The finding of anti-Ia(p28,33) binding to AML as well as ALL cells was unexpected and intriguing. Previously similar 'anti-B' cell sera were thought to be leukemia-specific; these studies probably gave inadequate consideration to cellular controls since several normal cell types are positive with anti-B

Table 2. Acute lymphoblastic leukemia associated antigen Expression in hemopoietic malignancy

Positive:	non-T,non-B ALL (85% of childhood ALL, 55% of adult ALL, total studied: 500) Thy-ALL (10%, pre-T) (12) pre-B-ALL (25) Ph^1+ AL/CML in 'L' blast crisis (30) AUL (50%, 15)
Negative:	Acute Myeloid, Monocytic, Myelo-monocytic leukemia (130) Chronic Myeloid Leukemia (25) Ph^1+ CML in 'M' blast crisis (55) Thy-ALL (90%, 110), B-ALL (8) Chronic Lymphocytic Leukemia (B,T (25)) B lymphomas, myeloma (30) Sezary syndrome (5) Erythroleukemia (7)

Taken from Roberts and Greaves, 1978, Greaves et al., 1977, and the unpublished observations of Greaves, Roberts and Janossy.
Number of cases studied in brackets

or 'Ia'-like sera (Greaves and Janossy, 1978). The presence of Ia^+(p28,33) molecules on AML cells is rationalized by correlation with the maturation status of these cells. This can be clearly demonstrated by an investigation of Ia antigen expression on normal myeloid precursors in vitro (M-CFUc) (see Moore, M., in this book). When Ia(p28,33) positive cells from normal bone marrow are selected on the Fluorescence Activated Cell Sorter using sterile techniques it can be shown that the majority of CFUc in man express the Ia antigen (Greaves et al., 1978; Janossy et al., 1978). It has also been shown that 'anti-B' cell sera and complement eliminate M-CFUc (Cline and Billing, 1977; Kaplan et al., 1978); although this latter approach is technically less satisfactory the result accords with expectations.

4. Levels of Maturation Arrest and 'Target' Cells for Leukemogenesis in Man

The phenotype described for leukemic cells and the corresponding level of maturation arrest does *not* necessarily identify the 'target' cell. A block to further maturation may occur at the level of 'target' cells (e.g. in some Avian leukemias, see Graf, this book). However, there is evidence that this need not be the case. This point is best exemplified by chronic myeloid leukemia (CML) which is a hemopoietic stem cell disorder associated with a specific chromosomal change [t(22Q$^-$): the Ph^1 or Philadelphia chromosome] (see papers by Rowley and Fialkow in this book). This Ph^1 positive clone is usually dominant in the granulocytic, monocytic, erythroid and platelet lineages even though overt leukemia is only usually evident in the granulocytic series. Attempts to identify the Ph^1 chromosome in PHA stimulated (T) lymphocytes have generally failed. This would appear to place the 'target' cell at the level of a myeloid stem cell with all hemopoietic options excluding lymphocytes. Recent evidence indicates, however, that in some cases at least the target cell might in fact be the pluripotential stem cell (Janossy et al., 1976). CML frequently enters an acute phase or 'blast crisis'; in a minority of cases the cells seen in this stage of disease resemble ALL cells which prompted Boggs to propose that this might indicate that CML was a pluripotential stem cell disorder (Boggs, 1974). Morphology, of course, can be misleading. Detailed phenotypic analysis of CML in blast crisis, however, supports the pluripotential stem cell thesis (Janossy et al., 1977a; Greaves and Janossy, 1978). Some blast crises are TdT positive (Sarin et al., 1976; McCaffrey et al., 1976; Hoffbrand et al., 1977) and more than 30 cases have been identified in our own series which share the cALL phenotype (i.e. $cALL^+$, Ia^+, TdT^+, $SmIg^-$, T^-, E^-, M^-; cf. Table 1 and Fig. 1) (Janossy et al., 1977a; Hoffbrand et al., 1977). The ALL and Ia antigens on Ph^1 positive cells are completely cross-reactive with those of Ph^1 negative ALL cells (Janossy et al., 1977a) and have the same general molecular characteristics (Sutherland et al., 1978; see above). Cell separation studies indicated furthermore that the Ph^1 chromosome was indeed present in the 'ALL' type cells in blast crisis (Janossy et al., 1978). We have suggested (Janossy et al., 1976; Greaves and Janossy, 1978) that the sharing of a common phenotype is indirect but compelling evidence for the

following two points: 1. That common ALL and some blast crises of CML have a maturation arrest at the same or closely-related level of early lymphoid lineage development and, 2. that, saving for second independent malignancies arising, these cases of CML *must* have been initiated in a pluripotential stem cell. It might be argued that since we have no instance (in over 100 cases examined) of a mature T or B cell phenotype arising in a CML that the credibility of this interpretation rests on the assumption that the cALL phenotype is exclusive to the lymphocyte lineages. At present this is difficult to establish unequivocally although we know that M-CFUc are ALL antigen negative (Janossy et al., 1978).

Recent observations have, however, provided direct evidence for involvement of the B cell lineage in Ph^1 positive CML. Out of 20 cases of blast crisis of CML investigated 3 had a 'pre-B' phenotype with cytoplasmic IgM (Greaves et al., 1979); similarly, the Ph^1 positive cell line (NALM-1) established from a case of CML in blast crisis also has a 'pre-B' phenotype.

This observation accords with recent data from Fialkow in which enriched B cell populations from a patient with Ph^1 positive CML appeared to be monoclonal with respect to Glu-6-P-D enzymes (see Fialkow, this book).

The developmental level of maturation arrest observed in leukemia is therefore very much a reflection of the competence of the dominant subclone to respond to maturation-linked signals – a critical factor which might alter radically as the disease progressively evolves (Nowell, 1976); in this respect CML in man finds an approximate parallel in the Friend virus-induced erythroleukemias of mice (Jasmin et al., 1976). Myeloma in man provides another similar instance. The myeloma Ig idiotypic antigens have been identified in pre-B cells in several patients (M. Cooper, personal communication) and, in one instance, on T as well as B cells (Preud'homme et al., 1977). These observations suggest that myeloma need not be a transformation of plasma cells but can be initiated in early B cells, a common T plus B progenitor or even earlier in the lineage sequence.

5. Leukemic Cell Lines

We have recently characterized a large series of leukemia and normal cell lines with respect to the antigenic markers discussed above (Table 1). These studies were carried out in collaboration with many different colleagues who established the original lines (see footnote to Table 3 and references therein). Table 3 is a summary of cALL antigen expression in cell lines; other details of the phenotypes can be found in the references given. The cALL antigen expression in cell lines parallels that observed in uncultured leukemic cells except that it is present in 2 B-cell lymphoma lines and unexpectedly in five out of six T-ALL lines (Minowada et al., 1978). One of the latter, MOLT-4, has no detectable cell surface ALL antigen but synthesizes and releases the 100K dalton polypeptide bearing the ALL antigenic determinant into the culture medium (Sutherland et al., 1978). The Ph^1 positive line NALM-1 established from a case of blast crisis of CML in a young girl has the cALL

Table 3. Acute lymphoblastic leukemia associated antigen Expression in leukemic cell lines

	Positive	Negative
1. *ALL*		
common ALL	3	0
Thy-ALL	5	1
1. *Ph^1+ CML-bc*	1	1
3. *B cell lines*		
B-ALL	1	3
Burkitt's Lymphoma	1	6
Non-Burkitt's Lymphoma	1	8
Myeloma	0	2
Normal B cells	0	20
4. *Others*		
Histiocytic lymphoma, Myelomonocytic leukemia, neuroblastoma	0	5

From Minowada et al., 1978, Rosenfeld et al., 1977, plus unpublished observations of Greaves, Nilsson and Schneider

phenotype characteristic of 'lymphoid' blast crisis (Minowada et al., 1977). In contrast the line K562 which was also established from Ph^1 positive blast crisis of CML lacks all of our markers including the 'myeloid' antigen. The latter antigen is, however, expressed on HL-60, a pro-myelocytic line (Boss and Greaves, unpublished; see also Gallo, this book) and U-937, a histiocytic lymphoma line (Roberts and Greaves, 1978). RAJI and NALM-6 are interesting lines in having a dominant 'pre-B' like phenotype, i.e. no or very little cell surface Ig but positive for cytoplasmic IgM (Greaves and Minowada, unpublished observations). It is important to note that all the ALL lines are difficult to establish and all those currently available are derived from patients in relapse. Also, it is probably very significant that all lines derived from malignant hemopoietic cells have aneuploid karyotypes. We hope to be able to use some of the ALL lines to investigate early lymphocyte differentiation in man. So far we have not been able to induce phenotypic alterations in these lines using ligands effective in other systems (e.g. DMSO, butyric acid) (Greaves and Verbi, unpublished observations).

6. Summation: Leukemic Cell Phenotypes and Hemopoietic Differentiation in Man

The analysis of an extensive series of acute and chronic leukemias of man with a panel of antigenic and enzymatic markers has provided us with some

insight into the heterogeneity of these leukemias and their biological properties. Irrespective of the outcome of our more academic objectives it is already clear that the subgroups of leukemias defined by these tests (e.g. in ALL and CML blast crisis) are distinct in terms of prognosis and therefore are important in providing useful aids in diagnosis and the allocation of patients to appropriate therapy groups.

The main conclusion to be drawn from this study to date is that the phenotypes appear to reflect normal gene expression which is appropriate for the developmental level at which maturation arrest has occurred. Leukemia-specific antigens, if they exist at all, have not been revealed by these studies. By definition the leukemia-associated membrane antigens are *differentiation* antigens whose expression is restricted in terms of cell lineage and maturation levels. It is important to recognise that some antigens, e.g. the cALL and p28,33 antigens may be restricted to infrequent hemopoietic precursors in particular lineages; their expression in leukemia might readily be misinterpreted as indicative of either fetal gene derepression (i.e. 'onco-fetal' antigens) or the presence of unique leukemic determinants.

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