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Comparative Antigenic Phenotypes of Normal and Leukemic Hemopoietic Precursor Cells Analysed with a "Library" of Monoclonal Antibodies

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A. Introduction

A dominant paradigm of cancer research is that alterations in the cell surface are of paramount importance to tumour cell behavior (Wallach 1978; Marchesi 1976). It is widely held that this is in part reflected in the regular expression of neo-antigens resulting from gene derepression [or "retrogressive differentiation" (Coggin 1978)], mutation (Baldwin 1974) or altered processing [e.g. glycosylation (Hakomori 1975)]. The search for novel antigens or other cell surface features of human tumour cells has an obligatory control demand which is frequently ignored or inadequately dealt with, i.e. that the appropriate cellular controls be analysed in parallel. Since most epithelial carcinomas and acute leukemias probably arise from tissue stem cells and, moreover, frequently have a maturation arrest imposed upon them, it should be self-evident that (a) many or most of the consistent phenotypic features of leukemic cells (and tumour cells in general) will be a reflection of their immature cell origins and (b) the significance of potentially unique biochemical or molecular features of tumour cells cannot be interpreted until we have access to normally infrequent tissue precursor cells.

The latter demand may be satisfied in the future by the development of new culture methods (see Dexter, this volume); in the meantime one of the most incisive approaches we have to the analysis of tumour cell phenotypes is the serological characterization of cell surface antigen expression on individual leukemic cells, particularly by monoclonal antibodies. The crucial advantages of leukemia in this context are that "equivalent" normal tissue is available in a physical form that is amenable to "cell surface" serology (i.e. single cell suspensions) and that stem cells and progenitor cells, whilst not morphologically recognisable, can be detected by functional assays in vitro. By the same token, acute leukemias offer an opportunity to discover antigenic and other characteristic marker features of hemopoietic stem cells which might be functionally relevant to the regulation of differentiation or at least be useful as "markers" for isolating these cells.

These arguments were in part developed in previous Wilsede symposia; here they are further explored with particular reference to two well-characterized cell surface glycoproteins – the gp 100 common ALL-associated antigen and the gp 28/33 Ia-like or HLA-DR antigens. In addition a systemic comparison of leukemic cells and their "presumed" equivalent normal counterparts using a panel of monoclonal antibodies is described.

B. The Terminal Transferase Positive "Lymphocyte" in Normal Bone Marrow has the Same Composite Cell Surface Phenotype as Common Acute Lymphoblastic Leukemia (cALL)

Rabbit antisera to non-T, non-B ALL have defined an antigen present on leukemic cells from 75% of children with ALL (common ALL) and on blast cells in some cases of AUL and CML in blast crisis (reviewed in Greaves and Janossy 1978; Greaves 1979a). The cell surface polypeptides (gp 100) reactive with anti-cALL have been isolated and characterized (Sutherland et al. 1978; Newman et al. 1981; Newman et al., this volume). Antisera with a similar if not identical specificity have now been produced by other laboratories

(Borella et al. 1977; Netzel et al. 1978; Pesando et al. 1979; Kabisch et al. 1979; LeBien et al. 1979), including a monoclonal antibody – J-5 (Ritz et al. 1980). Some of these sera, including the monoclonal J-5, also appear to precipitate a cell surface glycoprotein of 95–100,000 daltons (Billing et al. 1978; Pesando et al. 1980); however, several of these authors were unable to find normal bone marrow cells reacting with their reagents and therefore concluded that the latter could be identifying an antigen(s) unique to leukemic cells.

We have documented elsewhere the evidence that the cALL antigen as detected by our particular rabbit antibodies is present on small numbers of "lymphoid" cells in normal bone marrow and particularly in regenerating marrows of pediatric patients (Greaves et al. 1978; 1980; Janossy et al. 1979). Furthermore, a gp 100 molecule can be isolated from these sources with anti-ALL sera (Newman et al. 1981 and this volume).

Although some one-third of cALL have a "pre-B" (μ chain positive) phenotype (Vogler et al. 1978; Brouet et al. 1979; Greaves et al. 1979), the majority express no markers of mature T and B cells and presumably represent hemopoietic precursor cells in maturation arrest (Greaves and Janossy 1978); whilst they are likely to be lymphoid, i.e. precursors committed to T and/or B lineages, this is not formally proven. These leukemias also express the nuclear enzyme TdT which can be identified by fluorescent antibodies (Bollum 1979). A small proportion of normal lymphoid cells in bone marrow (as well as most cortical thymocytes) contain TdT (Bollum 1979); this enzyme, therefore, provides a very convenient single cell marker against which cell surface phenotype can be analysed. We reported previously that the TdT positive cell in normal bone marrow expressed the cALL and Ia-like antigens but not T cell antigens or Ig (Janossy et al. 1979). We have now assessed the composite antigenic phenotype of TdT-positive marrow cells using an extensive library of monoclonal antibodies (Greaves 1981a, b). The results (Table 1) indicate that the majority of TdT positive cells in bone marrow have a cell surface phenotype that is a replica of that seen in common ALL (Greaves 1981a, b; Greaves and Janossy 1978) and which includes no *exclusive* markers of either non-lymphoid lineages or mature T and B cells. The antigenic determinant detected by monoclonal PI153/3 which is present on most normal TdT-positive cells is, however, present on normal B cells as well as pre-B cells and cALL (Greaves et al. 1980). It should also be noted that the majority (90%) of TdT-positive cells in normal bone marrow do not express any of the T lineage antigens detected by the OKT series of monoclonals, including those that are reactive with some or most TdT-positive T-ALL (Reinherz et al. 1979a, 1980). An exception to this is OKT10 which, though reactive with most T-ALL (Janossy et al. 1978a), is also present on the majority of cALL and AML (Greaves et al. 1981).

Of 25 marrows analysed (donors 2–41 years) with monoclonal (J-5) anti-ALL, 21 showed positive reactivity on 2%–39% positive cells. This was variable in intensity but occasionally quite bright (Fig. 1a). There was a high degree of concordance with the TdT-positive cells (Table 1, Fig. 1c) in pediatric samples as previously reported with rabbit antisera (Janossy et al. 1979). Since monoclonal J-5 gives completely concordant reactivity pattern on more than 200 leukemias assessed (Ritz et al. 1980; M.F. Greaves and J. Ritz, unpublished work) and co-redistributes on the cell surface with rabbit anti-ALL (Fig. 1d), then the simplest explanation is (a) that it can recognise the same structure (though possibly not the same determinants) as rabbit anti-ALL and (b) that this structure, or one similar to it [since a family of gp 100 molecules may exist (Pesando et al. 1980)], is present on normal TdT-positive lymphoid cells in bone marrow. More detailed biochemistry is now required to determine the degree of similarity between the gp 100 molecules from cALL and normal bone marrow cells.

Another monoclonal antibody reactive with cALL has recently been described [BA-2 (Kersey et al. 1981)]. In contrast to J-5 and rabbit anti-cALL, this antibody appears to identify a p 24 structure; it is also present on a small number of normal bone marrow cells.

This analysis indicates therefore that the composite antigenic phenotype of cALL mirrors that of a normal (TdT^+) cell type in bone marrow. We presume therefore that (a) these determinants are most likely normal gene products of hemopoietic precursors that *continue* to be co-ordinately expressed in leukemia and (b) that the $cALL^+$ TdT^+ normal cell which is restricted to bone marrow (Greaves et

Table 1. Monoclonal antibody reactivity with TdT-positive bone marrow lymphocytes and cALL[a]

Selectivity	Designation	Ref.	Reactivity with TdT-positive cells in bone marrow	Reactivity with cALL
HLA associated:				
1. HLA-ABC "framework"	W6/32	Brodsky et al. (1979)	+(85–95%)	+>95%
	PA 2.6			
2. HLA-DR "framework"	DA2			
	OKI-1			
3. β_2 microglobulin	EC3			
	BB5			
T lineage associated:				
1. "Pan"-T	OKT11	[b]		
2. Mature T	OKT1	Reinherz et al. (1979b)	–	–
	OKT3	Kung et al. (1979)		
	L17 F12	Levy (to be published)		
3. Functional subset				
(a) "Suppressor" T	OKT8	Reinherz et al. (1980)		
(b) "Helper" T	OKT4	Reinherz et al. (1980)		
4. Intrathymic subset	OKT6	Reinherz et al. (1980)		
	NA134	McMichael et al. (1979)		
5. Thymic associated[c]	OKT9	Reinherz et al. (1980)		
	OKT10	Reinherz et al. (1980)	+(50–95%)	+
B lineage associated:				
1. Pan-B	FMC1	Brooks et al. (1980)	–	–
2. Pre-B, Pan-B/Neural	PI153/3	Greaves et al. (1980)	+(45–90%)	+
		Kennett and Gilbert (1979)		
Common ALL/lymphocyte progenitor associated:				
	J-5	Ritz et al. (1980)	+(45–95%)	+
Other non-lymphoid lineages:				
1. Monocyte/Granulocyte	OKM-1	Breard et al. (1979)	–	–
2. Erythroid:				
Glycophorin A	LICR.LON/R10	Edwards (1980)		
Band III	1/6A	Edwards (1980)		
3. Platelet[d]	AN51	McMichael (to be published)		

[a] All monoclonals have been tested on 2–5 normal bone marrow suspensions and a minimum of 20 cALL

[b] A pan-T monoclonal antibody, reactive with the sheep erythrocyte receptor on T cells (W. Verbi, M. F. Greaves, G. Janossy, P. Kung and G. Goldstein in preparation)

[c] OKT9 reacts with approximately 10% of thymocytes and OKT10 with all thymocytes. However, neither monoclonal is T-lineage specific. OKT9 reacts with the receptor for transferrin which is ubiquitous in distribution and associated with cell proliferation (Sutherland et al. 1981). OKT10 reacts with almost all non-lymphoid acute leukemias (Greaves et al. 1981)

[d] Reacts selectively with platelets and possibly with megakaryocytes. –, less than 2 cells per 100 positive. With the exception of OKT6 and NA134, all monoclonals stained some cells in the marrow suspension tested. All reagents were titrated on the Fluorescence Activated Cell Sorter (FACS-I) to determine the maximum dilution giving 2× saturation on known positive control cells. Binding of mouse monoclonal antibodies to viable cells in suspension was followed by the addition of affinity-purified goat antibodies to mouse Ig which had been cross-absorbed with insolubilized human Ig digested with pepsin to give a $f(b)_2$ preparation and labelled with rhodamine isothiocyanate. Cells were then smeared (cytospin), fixed in methanol and stained with affinity purified rabbit anti-TdT followed by fluorescein isothiocyanate-labelled and affinity-purified goat $f(ab')_2$ anti-rabbit Ig

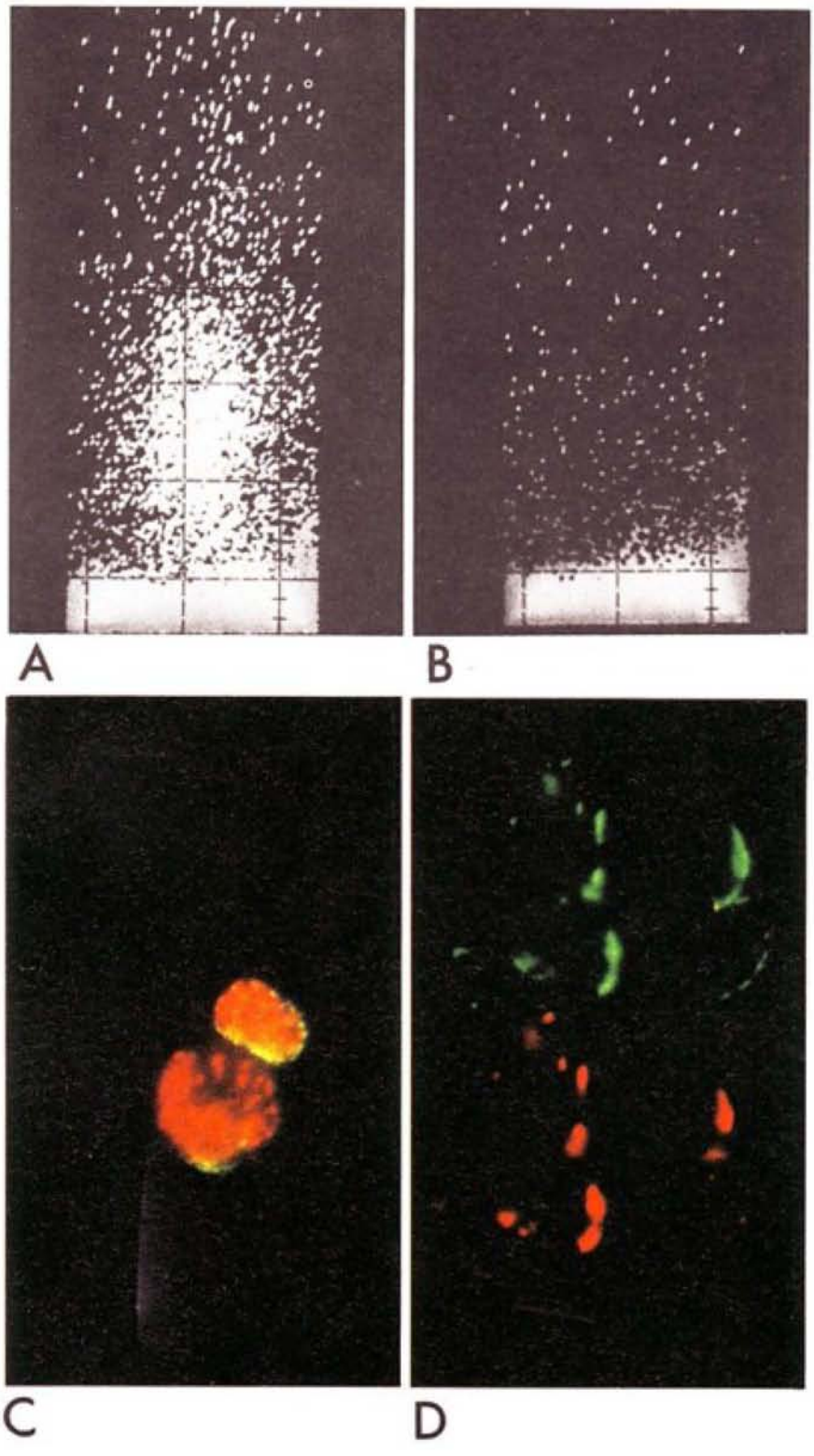

Fig. 1. Reactivity of normal and leukaemic cells with monoclonal J-5 anti-ALL (gp100) antigen. **A,B.** FACS analysis. Vertical axis, relative fluorescence intensity; horizontal axis, relative cell size (light scattering). Uninvolved bone marrow from a child with rhabdomyosarcoma was stained with J-5 anti-ALL (A) or control mouse ascites Ig (B). **C.** Normal paediatric bone marrow cells stained (in suspension) with monoclonal J-5 anti-cALL (gp100) plus (after cytospin preparation and fixation) rabbit anti-TdT. Cell surface stains green/yellow for the cALL antigen and nucleus red/orange for TdT. **D.** ALL cell line (Nalm-1) cells stained first with rabbit anti-cALL (gp100) under capping conditions rhodamine labelled goat anti-rabbit Ig added at 37° for 30 mins. Cells were then kept in the cold (4°) with sodium azide and stained with mouse monoclonal J-5 anti-ALL followed by fluorescein labelled goat anti-mouse Ig. Field of 4 cells was photographed using filters for rhodamine (upper half of picture) then moved slightly to re-expose same photograph frame for fluorescein (lower half of picture). Note complete co-incidence of red and green images indicates co-redistribution of the rabbit and mouse antibodies

al. 1979; Janossy et al. 1979) is either the major "target" population for cALL and/or represents a post-target developmental level of maturation arrest in ALL [as evidenced for example by cALL blast crises of CML (Greaves and Janossy 1978)].

C. The Cellular Selectivity of HLA-DR Expression in Leukemia Parallels Its Presence on Hemopoietic Progenitor Cells of the Myeloid and Erythroid Lineages

The Ia-like, p28,33 or HLA-DR antigens (Moller 1976) are present on pre-B cells, B lymphocytes, a T cell subset macrophages and different types of epithelia, e.g. thymic, intestinal and lactating mammary. Plasma cells, thymocytes and most T cells have no demonstrable cell surface HLA-DR. Hetero-antisera and allo-antisera to these molecules react with B cell leukemias (e.g. CLL) as well as almost all cases of non-T ALL (Greaves and Janossy 1978). More surprisingly, AML (Schlossman et al. 1976; Janossy et al. 1978b) and CML in "myeloid" blast crisis (Janossy et al. 1977) were found to express HLA-DR or Ia-like antigens. These observations have now been rationalized by reference to HLA-DR expression on normal hemopoietic precursors. Thus some normal immature myeloblasts may express Ia-like antigens (Ross et al. 1978; Winchester et al. 1977). CFU-GM activity in vitro can be inhibited by pretreating with anti-Ia-like reagents and complement, (Koeffler et al. 1979; Moore et al. 1980) and CFU-GM can be positively selected on the fluorescence-activated cell sorter (FACS) using rabbit antibodies to the p28,33, Ia-like or HLA-DR polypeptide complex (Janossy et al. 1978a).

These observations have now been confirmed and extended using a monoclonal antibody [DA2 (Brodsky et al. 1979)] to a monomorphic or conserved determinant of HLA-DR. Table 2 lists the leukemias that show reactivity with this antibody. Acute myeloblastic leukemias are usually but not invariably positive with anti-HLA-DR, whereas acute promyelocytic and chronic granulocytic leukemias are negative, which further emphasizes the inverse association between HLA-DR expression and granulocytic maturation.

Notice that erythroleukemias are consistently HLA-DR negative (Table 2). This observation is of some importance in relation to two other reported observations: (a) that both BFU-E and CFU-E can be inhibited by rabbit anti-p28/34 and complement (Moore et al. 1980; Winchester et al. 1978) and (b) that rabbit anti-glycophorin may detect "cryptic" early erythroid leukemias which would otherwise escape this differential identification (Andersson et al. 1979, 1980 and see also Andersson, this volume).

We have used both "conventional" antisera to glycophorin and a monoclonal antibody [LICR.LON.R10 (Edwards 1980)] to screen large numbers of different leukemias. To date we have detected three cases of glycophorin positive acute leukemias that were not overtly

Table 2. Reactivity of different leukemic cells with monoclonal anti-HLA-DR (DA2)[a]

ALL:	
cALL	201/203
T-ALL	0/53
"Null"-ALL	38/38
B-ALL	5/5
CGL-blast crisis ("L" type)	15/15
Chronic l. leukemias:	
B-CLL	26/26
B-PLL	5/5
T-PLL	0/6
T-CLL	1/7
T-Sezary	1/5
B-hairy cell leukemia	4/4
Myeloma/Plasma cell leukemias:	0/4
Myeloid leukemias:	
AML	83/110
AMML	20/28
AMonL	8/9
CGL	1/26
CGL blast crisis ("M" type)	17/21
APML	0/6
Erythro-leukemias:	0/11

[a] L, lymphocytic; ALL, acute lymphoblastic leukemia; CGL, chronic granulocytic leukemia; PLL, prolymphocytic leukemia; CLL, chronic lymphocytic leukemia; AML, acute myeloblastic leukemia; AMML, acute myelo-monocytic leukemia; AMonL, acute monocytic leukemia; APML, acute promyelocytic leukemia; "L" type, lymphoid ($TdT^+/cALL^+$) variety of blast crisis; "M" type, myeloid or non-lymphoid ($TdT^-/cALL^-$) variety of blast crisis

erythroid. Two were CML in blast crisis and one was a child with poorly differentiated acute leukemia (Greaves 1981a). In these cases a proportion of cells also reacted with monoclonal and polyclonal anti-HLA-DR; however, double labelling showed that glycophorin and HLA-DR were present almost exclusively on different cells.

To explore further the significance of erythroleukemic phenotypes in relation to normal early erythroid differentiation we have labelled normal bone marrow cells with various monoclonal antibodies, separated positive and negative cells under sterile conditions using the FACS and assayed for BFU-E and CFU-E activity. The details of these results are published elsewhere (Robinson et al. 1981) and summarized as a 'model' diagram in Fig. 2. BFU-E are predominantly HLA-DR$^+$, HLA-ABC$^+$, and glycophorin$^-$; CFU-E are predominantly HLA-DR$^-$, HLA-ABC$^+$, and glycophorin$^-$. All morphologically recognisable erythroid cell precursors are HLA-DR$^-$, HLA-ABC^{+or-}, and glycophorin $^{+or-}$. All erythroid progenitors (BFU-E and CFU-E) were in addition reactive with monoclonal anti-blood group A (in an A$^+$ donor) but unreactive with OKT1, OKT11 and J-5 (see Table 1). As an incidental observation in these experiments (since the cultures were all set up with erythropoietin) we noted that CFU-GM and CFU-Eo when present also localized predominantly in the HLA-DR$^+$, HLA-ABC$^+$, glycophorin$^-$ population.

These observations, therefore, establish as directly as is currently possible that HLA-DR antigens are indeed expressed on committed hemopoietic progenitor cells [although they may be absent from pluripotential stem cells (Basch et al. 1977; Moore et al. 1980) and raise the possibility that cell interactions involving HLA-DR or Ia-like antigens might play a role in early hemopoiesis as well as in immune responses (McDevitt 1978).

Since both covert and overt erythroleukemias are glycophorin$^+$, HLA-DR$^-$ we can place their likely dominant maturational arrest position close to the post-CFU cells. However, erythroleukemia can almost certainly originate in a pluripotential progenitor cell, since it regularly involves a granulocytic component or may indeed occur in Ph^1 positive CML. These studies with monoclonal antibodies confirm that glycophorin may provide a useful marker for cryptic early erythroleukemia (Andersson et al. 1979, 1980) but also indicates that many more HLA-DR$^+$ or HLA-DR$^-$ acute leukemias corresponding to BFU-E or CFU-E, respectively, might exist but remain undetected as such since no exclusive marker for these early erythroid cells yet exists.

D. Conclusions

Detailed serological analysis of leukemic cell surfaces using both conventional and monoclonal antibodies indicates that acute leukemic cells have composite antigenic phenotypes that appear to correspond to their lineage affiliation and "position" of maturation arrest. If leukemia specific antigens exist then they are not readily revealed by this type of investigation. Although leukemic cells appear to show a remarkable fidelity of phenotype, the degree

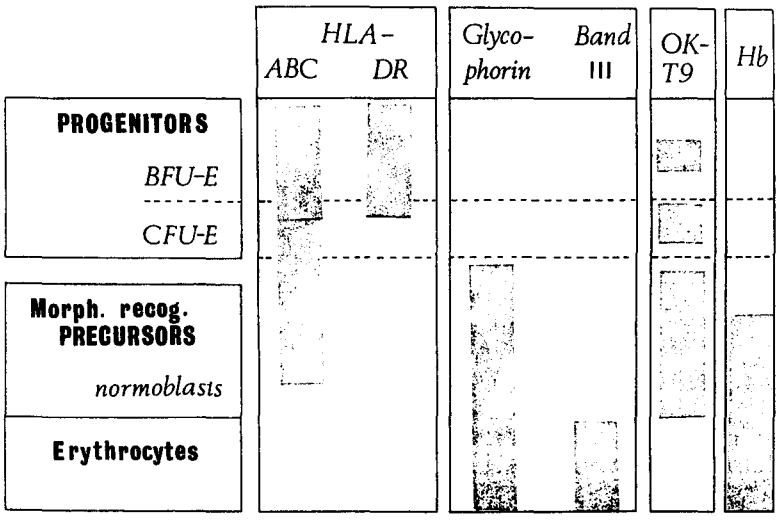

Fig. 2. Patterns of cell surface antigen expression during erythroid differentiation. *Hb*, haemoglobin

to which this is an exact replica of the normal counterpart is still open to question. Subsequent analyses with monoclonal antibodies could identify novel antigens perhaps restricted to individuals or small subsets of paients or occurring in association with particular chromosomal alterations (Rowley 1978). Karyotypic data suggest that gene dosage effects might have a critical bearing on leukemia (see G. Klein, this volume) and, similarly, quantitative rather than qualitative alterations in cell surface antigens might be important.

Finally, some putative anomalies in antigenic expression are encountered in studies on human leukemic cells (Shumak et al. 1975; Bradstock et al. 1980; Greaves 1979c, 1980), although it can be ruled out that these examples also reflect our ignorance of the heterogeneity of normal immature cell phenotypes. Since acute leukemia is generally regarded as a fairly high grade malignancy, it is of some interest to find that cell surface phenotypes are conserved or only marginally altered, suggesting an analogy with "minimally-deviated" hepatomas (Potter 1978). This permits some speculation about the contribution of the cell surface in malignancy (Greaves 1979b) and, as shown above, reveals characteristics of normal hemopoietic progenitors.

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