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Immunobiology of Lymphoid Malignancy

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A. Introduction

The study of 'membrane markers' in human leukaemia has now been in progress for a decade. Starting from the initial observation of L. Borella and colleagues at St. Judes on the sub-types of ALL [1] a wealth of data has accumulated particularly over the past few years with the introduction of monoclonal antibodies. Now is perhaps a good time to appraise the impact of these efforts and the implications for future research on leukaemia.

As Seligmann, Kersey, myself and others have emphasised on many occasions, the single most fruitful product of this activity has been the appreciation of how the cellular heterogeneity of lymphoid leukaemia and lymphoma mirrors stages of normal differentiation. This clearly arises as a consequence of three salient features of haemopoietic malignancy: the restricted or clonal origin [2], the imposition of maturation arrest, and the broad conservation or fidelity of a qualitatively normal phenotype [3].

The immunological and enzymatic definition of leukaemic cell phenotypes in relation to their normal counterparts has direct relevance to clinical problems of differential diagnosis, patient monitoring and variable prognosis [4]. Immunological features of ALL subgroups for example are linked to known prognostic features (e.g. high white cell count in T-ALL) and not surprisingly, therefore, show a strong correlation with the outcome of chemotherapy [1, 4–6]. Combinations of markers (e.g. cell surface antigens and nuclear terminal transferase [7]) offer the possibility of monitoring leukaemia and detecting residual, minimal or re-emerging extramedullary disease (i.e. CNS or testis).

The application of a panel of monoclonal antibodies has been routinely applied in my own laboratory for a national immunodiagnostic service over a number of years. It is difficult to determine precisely how useful such a service is; however, I estimate that the phenotypic data are essential in something like 15% of cases and are useful or supporting in many more (perhaps the majority). All of this is clear and undisputed; I would rather emphasise the broader and more substantial impact which I believe these studies should have.

Firstly, they provide a rational, biological framework for attempts to improve the efficacy of therapy either by more selective or 'tailored' allocation of particular regimes to defined leukaemic subgroups or by exploiting the biological information to design new or more radical strategies, e.g. monoclonal antibody elimination of leukaemic cells, selective enzyme inhibition. Secondly, they provide an essential framework for pursuing the molecular basis of haemopoietic malignancy. Since cellular oncogenes (or their viral homologues) are probably limited in number and have some important function in regulating normal differentiation and/or proliferation, it is of some importance to search for these genes and the expression and function of their products in the context of particular leukaemic subtypes and their normal counterparts; this is indeed already happening (see papers by F. Wong-Staal and M. A. Lane in this volume).

Some of the above points can be emphasised with reference to the biology of ALL.

B. Heterogeneity and Origins of ALL

Acute lymphoblastic leukaemia can be dissected in a number of subgroups with exclusive, composite phenotypes, which correlate with prognosis [4]. More recently, the use of monoclonal antibodies and immunoglobulin gene probes and the study of maturation induction in vitro has further elucidated the nature of ALL cells. It is now clear that ALL consists of two broad subtypes, both of which originate in lymphocyte progenitors (Table 1); one is 'pre-T' or equivalent to thymic precursors of mature T cells; the other, more common, variant is 'pre-B' or equivalent to B-cell progenitors and precursors in bone marrow. Within these two categories subtypes can be defined which broadly reflect sequential stages of maturation within the 'early' compartments of these two distinct cell lineages [8–10).

Table 1. Biological features of two ALL subtypes

	B precursor-ALL	T precursor-ALL
Dominant phenotype[a]	TdT^+ $HLA\text{-}DR^+$ T^- B^+ $cALL^+$ $Hex\text{-}I^+$	TdT^+ $HLA\text{-}DR^-$ T^+ B^- $cALL^-$ $Hex\text{-}I^-$
Ig genes	$\mu \pm \kappa/\lambda$ re-arr.[b]	No or minimal re-arr.
Growth fraction	Low	High
Karyotype	Hyperdiploidy common	Pseudodiploidy common
Likely cellular origin	Bone marrow B-lineage progenitor or stem cell	Marrow or thymic (subcapsular) T-lineage progenitor or stem cell
Diagnostic subtypes	Common ALL Pre-B ALL Null-ALL	T-ALL
Alternative diagnoses	AUL Ph^{1+} ALL NHL (rare)	T-NHL

[a] Serologically defined cell surface antigens or intracellular enzymes – terminal deoxynucleotidyl transferase and hexosaminidase isoenzyme I (plus charge variants of other acid lysosomal hydrolases: [42])

[b] Ig genes (e.g. V, D, J, μ heavy chain) re-arranged from germ line configuration [41]

Detailed studies on the antigenic phenotypes of these leukaemias provide no evidence for qualitatively aberrant gene expression or for a progenitor cell shared by and exclusive to the T and B lineages. Thus, ALL cells do not express glycophorin [11] or other restricted non-lymphoid markers; neither do they show concurrent expression on single cells or within a single leukaemic clone of markers unique to T and B cells. The 'pre-T' and 'pre-B' categories are also consistent features and although individual markers may change in relapse [12] there is no shift between these two subtypes during malignant progression in individual patients [3]. Normal counterparts of the ALL subtypes with qualitatively similar phenotypes (excluding karyotype) can be found in bone marrow [9, 13] and thymus [8, 10].

It is of some interest to note that whereas malignancies of lymphocyte precursors occur predominantly in children and young patients, malignancies of mature lymphoid cells (leukaemia, lymphoma, myeloma) are almost exclusively adult diseases [13a]; one interpretation of this correlation and the similarly striking age associations of other cancers (e.g. neural tumours versus epithelial carcinomas) is that they are a reflection of cell populations (stem cells?) at risk through proliferative demand at various stages of early development or during prolonged function (and turnover) in adult life.

The simplest interpretation of this descriptive data is therefore that ALL can originate in progenitor cells of either the T- or B-cell lineage and invariably suffers from the imposition of maturation arrest with the conservation of phenotype 'appropriate' for the particular stage of differentition in which the leukaemic cells become frozen or stabilised. Whilst I believe this general conclusion to be manifestly

therapy revert to CGL [18]. It is important to note that whereas B-cell progenitor ALL (e.g. common ALL) is curable with chemotherapy, blast crises manifest in this cellular compartment are not, although as expected they may achieve short-term remissions with steroids [19]. This sharp distinction provides an excellent example of the importance of "target cell" biology for understanding clinical outcome and developing appropriate alternative therapeutic strategies (e.g. marrow transplants for Ph^1-positive leukaemia).

4. ALL of either B or T progenitor type may not be diagnosed haematologically as ALL. Thus the majority of those rare (~5%) acute leukaemias which haematologists consider to be acute undifferentiated leukaemia are usually identifiable as ALL subtypes or more rarely as immature myeloid cells [4, 20]. Paediatric cases diagnosed as non-Hodgkin lymphoma may also belong or at least be very closely related to the two major subtypes of ALL. Conversely, not all cases diagnosed as ALL may be bona fide ALL. Thus, B-ALL is probably a misnomer; this relatively mature B-cell leukaemia probably represents a rapidly disseminating lymphoma [4, 21]. Rare cases of newborn acute leukaemia diagnosed as ALL may in fact be 'cryptic' erythroleukaemias as assessed by studies with monoclonal antibodies including anti-glycophorin [11, 22].

5. The maturation arrest imposed in ALL may be reversible, at least partially in vitro. Thus, some T-ALL cell lines can be induced by phorbol ester (TPA) to irreversibly modulate their composite phenotype from that of an immature or thymic variety to that of a mature T-cell subset [23, 24]. We and others have also been able to modulate the expression of TdT and cell surface antigen in B-cell progenitor ALL, although in our experience Ig synthesis cannot be induced in Ig^- ALL despite the presence of re-arranged μ chain genes. Our interpretation of this is that in leukaemia and in normal B-cell differentiation these recombinational genetic events are inefficient, with most clones failing to achieve a productive or functional re-arrangement.

The observation that maturation arrest in ALL is reversible as demonstrated previously with other leukaemias (e.g. Friend virus erythroleukaemia and myeloid leukaemia in rodents, avian erythroleukaemia and in some human leukaemic cell lines, e.g. HL-60, K562) carries the important corollary that maturation arrest, a central "lesion" in acute leukaemia, is a regulatory defect which, although having a genetic, inheritable basis, is reversible in its phenotypic consequences.

C. Is the Conservation of Phenotype Telling Us Anything Interesting About Leukaemic Cells?

It could be argued that since malignancy involves rare genetic events, it is to be expected that these will not have catastrophic effects on a cell's pattern of gene expression and that the broad fidelity of phenotype observed in ALL is (a) just what we would expect, and (b) boring and of no relevance or even downright misleading with respect to the central issue of what distinguishes a leukaemic cell from normal. Furthermore, it can always be that the 'critical' gene products in leukaemia are not those which we rather arbitrarily elect to study (so far) and that a more appropriate screen would reveal distinct, qualitative and consistent differences between leukaemic cells and their normal counterparts. These are not unreasonable views and I am surprised that they are not made more often.

I have favoured a different view initially because it was more interesting and subsequently because I believe it is supported by data. That is that the expression of qualitatively normal phenotype or pattern of gene expression is an integral and essential feature of most if not all leukaemias and other malignancies. Qualitative abnormalities (e.g. new or lost antigens, altered glycolipids, altered drug recognition) may occur and indeed have some selective advantage with malignant progression and treatment; however, they need not be considered as essential components of the malignant state. In the context of ALL, therefore, and as suggested some years ago [25, 26] a qualitatively normal lymphoid progenitor cell phenotype *which is normally only transiently expressed on proliferating cells* is quite compatible with leukaemic

Table 2. Structure, genetics and function of ALL-associated membrane proteins identified by monoclonal antibodies

	Monoclonal antibody (ref.)					
	J-5 [31]	BA-2 [34]	DA-2 [39]	OKT9 [14, 37]	OKT10 [37]	OKT11 [38]
Structural features:						
General	Single polypeptide ~ 100K [32, 33]	Single polypeptide ~ 24K	Two non-covalently linked polypeptides 28K (β), 33K (α) + intracellular 30K pp (HLA-DR)	Two s-s linked polypeptides ~ 90K [14, 37]	Single polypeptide ~ 40K [37, 38]	Single polypeptide ~ 40K
Glycosylated	+	±	+	+	+	+
pI	5.2 [33]	7.3 [35]	α: 5, β: 7[a] [36]	5.0 [28]		
Peripheral (p) or integral (I), transmembrane (t)	p	p	I.t.	I.t.	I	
Genetics: (chromosomal control)	?	12[b]	6	3 [29]	4[b]	
Function:	?	?	Cell interactions	Transferrin receptor [14] NK 'target'? [30]	?	Receptor for sheep erythrocytes[c] [38]

[a] Mean value; multiple spots observed with variable positions reflecting allelic polymorphism
[b] Katz, Povey and Greaves, unpublished observations
[c] Natural, physiological function unknown

cell behaviour and only requires that the genetic change provoking clonal selection effectively uncouples proliferation from maturation.

This view accords with recent molecular studies which reveal the central role of normal genes (*c-onc*) or their inserted viral (*v-onc*) homologues which may facilitate clonal advantage via amplification or excessive promotion ([27] and various papers in this volume). There is no evidence to date that qualitatively altered gene products are involved[1]. Much emphasis therefore rests on quantitative aspects of *c-onc* expression. Even this phenotypic distinction between leukaemic and normal cells could be small or perhaps only evident in the time frame, i.e. equivalent normal cells may express similar levels of *c-onc* gene products but only transiently.

D. Epilogue

Several of the ALL-associated membrane antigens have now been biochemically characterised and their control mapped to particular chromosomes (Table 2).

[1] An important example of such an alteration has however recently been reported [43]

Whether any of these proteins has any important regulatory role in differentiation or are even *c-onc* gene products is at present unknown. One of these structures does have a definite function. The monclonal antibody OKT9 identifies the transferrin receptor [14]; this observation has enabled rapid progress to be made in the biochemical studies of this receptor [28] and also facilitated the mapping of controlling (presumably structural) genes to chromosome 3 [29]. We have also suggested that the transferrin receptor may serve as a common 'target' structure on malignant and normal cells for so-called natural killer (NK) cells [30].

There are still many gaps in our understanding of lymphoid malignancy and of normal lymphopoiesis. Compared with myelopoiesis for example (see paper by Metcalf in this volume) we have little insight into soluble regulators of early lymphocyte development. Despite these limitations lymphoid malignancy in humans provides, I believe, an excellent example of a disease whose molecular, cellular and clinical complexity can be best understood in relationship to normal cellular differentiation.

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