Review article

Biochemical enzyme analysis in acute leukaemia

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SUMMARY This report summarises the current knowledge regarding the clinical utility of biochemical enzyme markers for both diagnostic and therapeutic purposes in acute leukaemia. The enzymes studied most extensively in this field are terminal deoxynucleotidyl transferase, adenosine deaminase, 5'-nucleotidase, purine nucleoside phosphorylase, and acid phosphatase, esterase, hexosaminidase isoenzymes. For each enzyme, the quantitative and qualitative characteristics in various immunologically defined subclasses of acute leukaemia are described. The quantitative evaluation of enzyme activities represents an adjunctive classification technique which should be incorporated into the multivariate analysis, the "multiple marker analysis." By qualitative characterisation pronounced heterogeneity of leukaemia subsets is uncovered. The application of 2'-deoxycytosine, a specific inhibitor of adenosine deaminase, and the potential usefulness of two other enzymes as targets for treatment with selective agents is discussed.

The concept that gene products expressed at certain developmental stages of normal cells can similarly be detected in leukaemic cells (which therefore seem to be "frozen" or "arrested" at this particular maturation/differentiation stage) is supported by the results obtained in enzyme studies. Besides their practical clinical importance for classification and treatment of acute leukaemias, biochemical enzyme markers constitute a valuable research tool to disclose biological properties of leukaemic cells.

In addition to traditional haematological methods such as morphology and cytochemistry (which have not become obsolete), highly specific techniques are required nowadays for the identification and sophisticated characterisation of malignant and normal haematopoietic cells. The recently acquired and tremendously expanded knowledge which has provided entirely new insights into the immunological, enzymological, cytogenetic, and functional aspects of leukaemia is due to some technical innovations. Above all, three advances in cell technology enhanced the progress in the understanding of the biology of leukaemic cells—namely, cell analysis and sorting performed by flow cytometry, the production of monoclonal antibodies by the hybridoma technique, and the tissue culture methodology for

maintaining permanent leukaemia-lymphoma cell lines.¹⁻³ The incorporation of many diverse disciplines such as morphology, cytochemistry, immunology, enzymology, chromosomal and cell kinetic studies, and others⁴ is needed for a careful analysis of the phenotypic profiles of leukaemic cells. The multidisciplinary approach, "multiple marker analysis,"³ has proved to be of considerable importance in leukaemia research. The fields of immunology and enzymology, especially when combined, seem to be particularly useful in phenotyping leukaemic cells and have provided a basis for a precisely defined, prognostically relevant and "biologically rational"⁵ subclassification of acute leukaemias.

In this report we summarise our own experiences with biochemical enzyme markers in conjunction with results of other authors, including recent studies of the potential therapeutic use of such

Table 1 *The seven best studied biochemical enzyme markers*

Enzymes	EC classification	Authors
Terminal deoxynucleotidyl transferase	2.7.7.31	McCaffrey <i>et al</i> ⁸
5'-Nucleotidase	3.1.3.5	Kramers <i>et al</i> ¹⁰⁹
Adenosine deaminase	3.5.4.4	Smyth and Harrap ¹¹⁰
Purine nucleoside phosphorylase	2.4.2.1	Meier <i>et al</i> ⁴⁸
Acid phosphatase	3.1.3.2	Li <i>et al</i> ⁶³
Esterase	3.1.1.1	Li <i>et al</i> ⁵⁷
Hexosaminidase	3.2.1.30	Ellis <i>et al</i> ⁷⁴

enzymes, and discuss the clinical importance of the data obtained.

Enzyme marker analysis

Leucocyte enzymes may be studied by either biochemical or cytochemical assays. There is a wide overlap in the practical application of these two main techniques, however, and even immunological methods are involved in enzyme marker analysis using antibodies raised against an enzyme and detected by immunofluorescence. Cytochemical studies have shown differing cellular distributions of a number of enzymes.⁶ The localisation of some enzymes at a cellular level has been improved by the use of cytochemical techniques at ultrastructural levels.⁷ Studies using biochemical assays examine either the total specific activity of several enzymes as the quantitative aspect in enzyme alterations or qualitative changes. The more subtle differences between cell types and lineages may be reflected in qualitative isoenzyme patterns.⁷

This report summarises the results of the most important enzyme marker studies published so far regarding acute leukaemia. Seven enzyme markers will be reviewed: these include terminal deoxynucleotidyl transferase (TdT), 5'-nucleotidase,

adenosine deaminase, purine nucleoside phosphorylase, acid phosphatase, esterase, and hexosaminidase. A synopsis of data regarding further descriptions of these enzymes is presented in Tables 1 and 2.

Interest in biochemical enzyme markers for diagnostic use in acute leukaemia stems from the discovery of high amounts of TdT in a case of acute lymphoblastic leukaemia (ALL).⁸ Subsequently, two more enzymes, adenosine deaminase and hexosaminidase, which besides TdT seem to be the most often applied and studied biochemical markers, have boosted the growing importance of biochemical enzyme marker analysis. These experiences with the clinical use of biochemical enzyme markers have stimulated interest in the application of enzymes as diagnostic tools.

Biochemical enzyme markers of leukaemic cells have been the topic of several recent reviews,^{7,9,15} which underlines the major progress in this young field. Instead of describing the enzymes according to merit in historical terms or importance, this review arranges them by functional aspects, presenting groups of enzymes and continuing to discuss the single enzymes.

Seven biochemical enzyme markers

Table 3 is a composite from our own results and the current knowledge in the published work, designed to permit comparison between these diagnostically relevant enzyme markers. This table provides a detailed account of the quantitative alterations of five enzyme markers as they were found in various subtypes of ALL, acute myeloblastic leukaemia (AML), and acute undifferentiated leukaemia (AUL); in addition, it gives an assessment of the incidence of raised TdT activities and of the isoen-

Table 2 *Biochemical characteristics of enzyme markers*

Enzymes	Location	Enzyme category	Catalytic function
Terminal deoxynucleotidyl transferase	Nucleus	Nucleotidyl transferase	Polymerisation of deoxynucleoside monophosphates without template (= DNA nucleotidyltransferase)
5'-Nucleotidase	Plasma membrane	Phosphohydrolase	Hydrolyses 5'-mononucleotides to corresponding nucleoside—for example, AMP, IMP to adenosine, inosine
Adenosine deaminase	Cytoplasm	Aminohydrolase	Irreversible conversion of adenosine, deoxyadenosine to inosine, deoxyinosine
Purine nucleoside phosphorylase	Cytoplasm	Pentosyl transferase	Conversion of deoxyguanosine, deoxyinosine to guanine, inosine
Esterase	Lysosomes, cytoplasm, plasma membrane	Carboxylic-ester hydrolase	Hydrolyses aliphatic, aromatic esters (non-specific = carboxylic esterase)
Acid phosphatase	Lysosomes	Phosphohydrolase (acid optimum)	Hydrolyses phosphate esters in acid environment
Hexosaminidase	Lysosomes	Glycosidase	Hydrolyses certain terminal glucose residues in glycoproteins (= β -N-acetylglucosaminidase)

Table 3 Distribution of enzyme activities in various leukaemic disorders

Enzymes	T-ALL	B-ALL	c-ALL	pre-B-ALL	Null-ALL	AML	AUL
Terminal deoxynucleotidyl transferase	90–100%↑	N	90–100%↑	90–100%↑	90–100%↑	5–15%↑	40–60%↑
5'-nucleotidase	↑	↓	↑	—	N	N or ↓	—
Adenosine deaminase	↑↑	variable	↑	—	↑	N or ↑	—
Purine nucleoside phosphorylase	↓	↓	N	—	↓	N or ↑	—
Hexosaminidase	neg	neg	75%	40%	45%	10%	60%

ALL = acute lymphoblastic leukaemia; AML = acute myeloid leukaemia; AUL = acute undifferentiated leukaemia. ↑ = increased; ↑↑ = greatly increased; ↓ = decreased; N = normal; — = not done.

zymes hexosaminidase I among these leukaemia subgroups.

TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE
Originally described in 1960 by Bollum,¹⁶ TdT is the most "potent" and used enzyme marker described to date. TdT is an unusual DNA polymerase, catalysing the polymerisation of deoxynucleoside monophosphates without template instruction.¹⁷ The normal distribution is restricted to cortical thymocytes and a minor population of bone marrow lymphocytes.¹⁶ TdT is usually shown by biochemical, radioimmunoassay, or immunofluorescent techniques.¹⁸

Since the report of McCaffrey in 1973, who discovered considerably raised TdT activity in a case of childhood ALL,⁸ blasts from several thousand patients with ALL have been assayed and reported as displaying high TdT activities in about 90% of the cases (85–95%).^{17 19–22} Only about 10% of cases (5–15%) of AML were TdT positive.^{10 23–26} Thus its virtual absence from normal mature haematopoietic cells and its presence at high levels in lymphoblasts has made TdT a valuable marker for lymphoid leukaemias, particularly in separating ALL from AML.¹⁴

High TdT activities were found in the great majority (90–100%) of patients with T-ALL, common ALL (c-ALL), pre-B-ALL and null-ALL.^{27–30} TdT has usually been negative in all patients with B-ALL.^{31–34} But 40–60% of cases of AUL expressed high TdT activities.^{28 35–36}

PURINE PATHWAY ENZYMES

5'-nucleotidase, adenosine deaminase, and purine

nucleoside phosphorylase are three sequential purine salvage pathway enzymes which have a certain impact on the functioning of the immune system.¹⁴ The inherited enzyme defects of 5'-nucleotidase, adenosine deaminase, and purine nucleoside phosphorylase are characteristic metabolic lesions causing or associated with congenital immune deficiency (Table 4).¹⁴ The simplified reaction sequence of this part of the purine metabolic pathway is seen in Fig. 1.

Enzyme activity quantitation is the biochemical approach used most often for these three enzymes to identify leukaemia subtypes.³⁷ Alterations in the enzymatic activity are reflected in pronounced decreases or increases in the specific activities of 5'-nucleotidase, adenosine deaminase, and purine nucleoside phosphorylase. The distributions of these three enzymes in subpopulations of normal thymocytes and peripheral lymphocytes have recently been described elsewhere.^{38–40}

5'-NUCLEOTIDASE

5'-nucleotidase activity was significantly decreased in cases of T-ALL and B-ALL compared with normal peripheral or bone marrow lymphocytes.^{37 41–45 117} The enzyme activity was raised in c-ALL^{42 44} and low or normal in AML.^{42 44 46}

ADENOSINE DEAMINASE

Adenosine deaminase activities were commonly increased in ALL compared with values in normal lymphocytes,⁴⁷ with the highest values occurring in T-ALL^{41 45 49–52} and raised levels in c-ALL and Null-ALL.^{42 53–54 114} The adenosine deaminase values of B-ALL cells were low, normal, or

Table 4 Congenital immune deficiency syndromes associated with purine pathway enzyme deficiencies

Enzymes	T lymphocytes	B lymphocytes	Immune deficiency syndrome	Original report
5'-nucleotidase	N	↓	Primary hypogammaglobulinaemia	Johnson <i>et al</i> ¹¹¹
Adenosine deaminase	↓↓	↓ or ↓ but impaired function	Severe combined immune deficiency	Giblett <i>et al</i> ¹¹²
Purine nucleoside phosphorylase	↓↓	N	Severe T lymphopenia	Giblett <i>et al</i> ¹¹³

↓ = decreased; ↓↓ = greatly decreased; N = normal.

Fig. 1 Example of a simplified cascade sequence catalysed by purine pathway enzymes. 5'-N = 5'-nucleotidase; ADA = adenosine deaminase; PNP = purine nucleoside phosphorylase; XOD = xanthine oxydase; AMP, ADP, ATP = adenosine mono-, di-, triphosphate.

increased.^{32 37 55} Patients with AML showed intermediate to high activities, which limits the value of adenosine deaminase assay in the differential diagnosis between ALL and AML.^{42 46 48 53}

PURINE NUCLEOSIDE PHOSPHORYLASE

Purine nucleoside phosphorylase concentrations were significantly lower in T-, B- and Null-ALL than in normal peripheral lymphocytes,^{41-42 45 54 56 117} whereas c-ALL cells showed activities within the normal range.⁴¹⁻⁴² Purine nucleoside phosphorylase values were significantly raised in AML compared with ALL, but not so when compared with normal peripheral blood cells.⁴²

LYSOSOMAL HYDROLASES

These include the enzymes acid phosphatase and hexosaminidase, which are localised in lysosomes and act at an acid pH (Table 2). Esterase represents a group of hydrolases with different characteristic features referring to specificity for substrates, sensitivity, or resistance to inhibitors, occurrence in certain cells, intracellular localisation, and cytochemical intracellular staining pattern.⁷ The carboxylic, also called non-specific esterase, has its pH optimum at neutral values.

Differences in qualitative terms between the various leukaemia types and subtypes have been described and recent isoenzyme studies of these three hydrolases showed some interesting isoenzymal changes.

ESTERASE

Polyacrylamide gel electrophoresis and electrofocusing studies showed the presence of distinct esterase isoenzymes and isoenzyme patterns which had some cell, cell status, and lineage specificity.⁵⁷⁻⁵⁹ As their isoenzyme patterns allowed a clear distinction bet-

ween acute myeloid and lymphoid leukaemias, these isoenzymes represent lineage markers.⁶⁰⁻⁶¹ Moreover, by the criterion of specific and selective inhibition with sodium fluoride, a band indicating a monocytic component in myelomonocytic leukaemias could be shown.⁶⁰ The incomplete expression of the total repertoire of esterase isoenzymes helped to identify certain developmental positions attained by the dominant malignant clone in cases of ALL.⁶⁰ When cases of c-ALL were tested for their isoenzyme profiles considerable heterogeneity within this subtype was discovered.⁶² Null-ALL cases did not display any esterase isoenzymes in one series.⁶²

ACID PHOSPHATASE

Acid phosphatase extracted from acute leukaemia cells has been separated into isoenzymes by means of polyacrylamide gel electrophoresis.⁶³⁻⁶⁶ One particular isoenzyme which was resistant to inhibition with tartrate was claimed to be specific for hairy cell leukaemia.⁶⁷⁻⁶⁸ By the refined method of isoelectric focusing differences in acid phosphatase profiles were seen in different ALL subtypes.⁶⁹⁻⁷² Isoenzymatic changes served to define specific stages of differentiation of the leukaemic blast cells within ALL subtypes. The progression in differentiation along the presumed developmental axis was paralleled by the increase in the incomplete repertoire of isoenzymes.⁷³ None of the acid phosphatase isoenzyme patterns, however allowed definite discrimination between these ALL subtypes; nor did they distinguish between myeloid and lymphoid lineages, in contrast to the above mentioned enzyme esterase.^{62 73}

HEXOSAMINIDASE

Hexosaminidase (β -N-acetylglucosaminidase) could be separated into its three isoenzymes (A, B, I) by ion exchange chromatography or isoelectric focusing/disc electrophoresis.⁷⁴ The abnormally high expression of hexosaminidase I (the intermediate band I) was noted in cases of c-ALL.⁷⁴⁻⁷⁵ This particular isoenzyme disappeared in remission and reappeared during relapse of the patients.^{13 70} The abnormally high peak of hexosaminidase I was not seen in T- and B-ALL, whereas it was found in most cases of c-ALL, half the cases of pre-B-ALL, Null-ALL, and AUL, and in a few cases of AML.^{7 29 62 76-77}

Enzyme marker analysis as an adjunctive classification tool

Classification of patients with acute leukaemia has advanced considerably over the past decade. Sub-

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groups that denote characteristic markers such as c-ALL, T-ALL, pre-B-ALL, and B-ALL have been identified within the main group ALL. The tremendous progress achieved in the subclassification is mainly due to the revolutionised techniques used in immunologically orientated leukaemia research. With the growing importance of enzyme marker analysis, essentially an offspring of the traditional discipline cytochemistry, the biochemistry of leukaemia research emerged as a valuable research and clinically relevant diagnostic tool. Cellular biochemical profiles complement the established markers and thereby enzyme marker analysis constitutes an ideal adjunctive classification technique. The combination of immunological and enzymological markers is already proving to be useful for defining and characterising lymphoid cell populations and for tracing developmental sequences. But, as the subject is more than complex and as leukaemic and normal cells cannot be specified by single characteristic markers, an adequate resolution of functional subsets requires multiparameter definition including examination of traditional features such as morphology and cytochemistry. In this multiple marker analysis³ enzyme marker studies play an important part for the purpose of further characterising cells.¹²

The highly improved classification techniques and the advanced knowledge on the relation between leukaemic cells and their position in haematopoietic cell differentiation has led to a reappraisal of classification schemes of leukaemias.⁷⁸ In turn, the marker identification in both leukaemia cell lines and fresh leukaemia has provided entirely new insights into the biology, immunology, and enzymology of both normal and malignant haematopoietic cells.³ The new information changed the models of schemes of the normal haematopoietic cell differentiation.³ Currently used classification systems of leukaemia are based mainly on classic features (morphology and cytochemistry) and modern marker studies (above all immunological and enzymatic criteria).^{3,79}

Enzymes seem to be useful as leukaemic markers to detect subclinical residual malignant cells and to monitor impending relapse. TdT testing is valuable in discovering the presence of small numbers of blast cells in extramedullary sanctuaries—for example, meninges or testes.^{14,80–81} The use of enzyme markers as prognostic determinants has not yet been fully exploited. Enzyme markers might give indices predictive for clinical behaviour and prognosis.¹⁵

The widespread availability of simplified assays and specified techniques for identification and quantitation of surface markers and enzyme alterations, both qualitative and quantitative, should facilitate

examination of these parameters in all patients with leukaemia. Detailed characterisation of leukaemic cells using multiple marker analysis should provide further important information about normal haematopoietic cell ontogeny and the processes entailed in leukaemia.

Enzyme marker phenotypes

A definitive marker combination, both immunological and biochemical markers, helps to identify the particular cell population.³ The presence of several enzyme markers together with the positive identification of some immunological features points toward a certain leukaemic subtype. Thus within the haematopoietic system, enzymes expressed in a lineage or stage restricted fashion or both may be used to map acute leukaemias (Fig. 2).

TdT is particularly valuable in separating acute lymphoid from myeloid leukemias.¹⁴ Otherwise, the main group AML did not show one distinct enzyme marker phenotype—for example, the quantitative measurement of the three purine salvage enzymes 5'-nucleotidase, adenosine deaminase, and purine nucleoside phosphorylase showed a substantial variation of the enzyme values; normal or low levels for 5'-nucleotidase and normal or high enzyme amounts for adenosine deaminase and purine nucleoside phosphorylase (Table 3).

Fig. 2 Enzyme marker profiles of acute lymphoblastic leukaemia (ALL) subtypes commonly defined by immunological markers (cALL-Ag = common ALL antigen; CyIg = intracytoplasmatic IgM; SmIg = surface immunoglobulin; E = Sheep erythrocytes rosette receptor; T-Ag = T lymphocytes associated antigens; TdT = terminal deoxynucleotidyl transferase; Hex I = hexosaminidase I. For other abbreviations see Table 1. TdT, ADA, 5'-N, Hex I: positive markers = increased enzyme levels. PNP: negative marker = markedly diminished.

The results obtained by studies of the above described enzyme markers suggest a biochemical heterogeneity within the range of AML cases; in other words, the group AML might consist of a number of subsets with quite different enzymatic features. Interestingly, these findings of the apparent biochemical heterogeneity of AML cells are consistent with results of immunological tests—that is, that AML represents, in terms of phenotypic profiles, a rather complex, “multi-coloured” group of acute non-lymphoid leukaemia subsets than a narrowly defined entity.

In contrast to the highly advanced identification and description of ALL subsets, a comparable analysis of AML patients has been initiated only in the past few years (except the FAB classification based merely on morphology and cytochemistry⁸²). A subclassification of AML into three subgroups by means of polyclonal antisera has been proposed.^{3,83} Characterisation of the blast cells in AML by monoclonal antibodies including the enzyme marker TdT provided new ways of testing these conclusions since for each subgroup variation in marker expression was noted.^{84–85} Further functional studies with both surface markers and biochemical parameters comparing these three or more AML subsets will certainly better define their differentiated state.

For ALL distinct enzyme marker phenotypes could be assigned to immunologically defined subsets (Fig. 2, Table 3). Distinct patterns of quantitatively measured enzyme activities were seen in T-, B-, common, null- and pre-B-ALL. T-ALL showed high values of TdT and adenosine deaminase and decreased values of 5'-nucleotidase and purine nucleoside phosphorylase, whereas hexosaminidase I was not found. B-ALL showed low purine nucleoside phosphorylase, low 5'-nucleotidase, and variable adenosine deaminase values; TdT and hexosaminidase I were negative. Pre-B-ALL showed positivity for TdT and hexosaminidase I. Common ALL and Null-ALL had a pattern of enzyme activity similar to that found in T lymphoblasts in so far as TdT and adenosine deaminase values were high; but, in contrast 5'-nucleotidase was significantly increased along with the expression of hexosaminidase I in c-ALL, whereas null-ALL had normal 5'-nucleotidase and low purine nucleoside phosphorylase values.

Qualitative ALL subset analysis of esterase, acid phosphatase, and hexosaminidase showed that the expression of isoenzymes and isoenzyme patterns may correlate with lymphocyte differentiation. This interpretation supports other studies which seem to indicate that lymphoid leukaemic differentiation is “frozen” at some specific developmental point (see below).⁸⁶

Enzyme inhibition—a new strategy in leukaemia therapy

Previous approaches to the biochemical exploration of enzyme functions in leukaemic cells have focused on the value of enzymes only for diagnostic purposes. Several recent observations suggest, however, that the selective inhibition of some of these enzymes represents another intricate aspect of the use of enzymes in leukaemia.

The hereditary deficiency of adenosine deaminase (Table 4), which is responsible for the conversion of adenine to inosine (Table 2), results in cell toxicity via accumulation of compounds located earlier in the metabolic pathway—for example, dATP in the case of adenosine deaminase.¹⁴ These findings served as the basis for pharmacological investigations of enzyme inhibitors in the treatment of leukaemia.

2'-deoxycytosine is a potent and specific inhibitor of adenosine deaminase which produces by its lymphotoxicity selective lymphopenia (Table 5).⁸⁷ In vitro tests showed that the combination of 2'-deoxycytosine and 2'-deoxyadenosine is highly toxic to T-ALL blast cells.^{88–89} In clinical studies the inhibitor was effective in obtaining remissions in most cases, even those resistant to conventional chemotherapy.^{90–93} A number of groups are currently examining the potential usefulness of 2'-deoxycytosine as a selective agent for the treatment of particular cases in controlled phase I-II clinical/pharmacological trials.^{94–99}

This drug design targeted against a “key enzyme”—for example, adenosine deaminase, on the basis that its activity is considerably increased in a given leukaemic population—can serve as an example of the proposed “enzyme (pattern) targeted chemotherapy”.¹⁰⁰

There is evidence suggesting that other key enzymes might be used as the targets of this new approach in treatment. Two further candidates are TdT and purine nucleoside phosphorylase (Table 5). Important relations exist between TdT and purine metabolic enzymes.⁴⁰ Moreover, results of in vitro studies showed that the inhibition of the activities of the specific enzymes TdT and purine nucleoside phosphorylase constitutes a lethal event to cells with high enzyme activities.^{101–102} The 6-anilino-uracil derivatives GW 17E, GW 18C, and GW 20DN were effective in killing cells by inhibition of TdT in experiments performed on animals and human cell lines.^{36,101} The guanosine analog 8-aminoguanosine was described as an effective inhibitor of purine nucleoside phosphorylase, both in vitro on cell lines and in intact lymphoid cells.¹⁰² α , β -methylene adenosine diphosphate has been reported to cause

specific inhibition of the activity of 5'-nucleotidase.¹¹⁵⁻¹¹⁶

Although the results of these still preliminary but promising studies suggest that these inhibitors could be both effective cytolytic agents and highly specific for some particular cells (for example, T lymphoblasts), the potential usefulness of these compounds or similar analogues in the treatment of leukaemia has yet to be further explored.

Role of enzyme markers in the concept of maturation arrest

The question arose as to whether certain isoenzymes reflect a characteristic, perhaps even specific, feature of the given leukaemic population or stem from the normal expression of cells otherwise only passing through this stage of differentiation. It seems that the leukaemic cells express these gene products (for example, the isoenzyme hexosaminidase I), which are normally found only during certain early phases of cell development, permanently because the blast cell development is blocked at this particular point. The detection of this isoenzyme hexosaminidase I would be comparable to taking one single frame out of a running film. Such theories have already been reported and emerged primarily from immunological studies comparing leukaemic cells and their supposed normal counterparts.

Evidence supporting the concepts of frozen differentiation,¹⁰³ maturation arrest,¹⁰⁴ and lineage fidelity¹⁰⁵ is provided by the recent studies using monoclonal antibodies to identify a large panel of immunological markers.⁵ No unequivocally leukaemia specific antigens have been described so far,^{3,5} which further emphasises the above models. Nevertheless, as it seemed unlikely that leukaemia differentiation always mimics the normal course of development precisely and the pathological lesions underlying the leukaemic process might affect such mechanisms, after careful testing many individual cases "a possible asynchrony of gene expression"⁷⁵ was noted. Likewise, the observation of highly raised activities of certain enzymes or the inappropriate occurrence of isoenzymes might be construed as a quantitative amplification of a normal process⁸⁶

or "the abnormal, deranged expression of normal gene products."¹⁰⁶

Thus "reprogramming of gene expression"¹⁰⁰ in leukaemic cells might be reflected in both quantitative and qualitative alterations.¹⁰⁰ The malignant program may be characterised by the capacity of the leukaemic cells for an escalation in expression of leukaemic properties—for example, an amplification of enzyme activity or an isoenzyme imbalance.¹⁰⁰

As an alternative to models of leukaemic processes that postulate that the normal differentiation programs are merely blocked stands the hypothesis that leukaemic programs are assembled abnormally—that is, programs are abnormal if an extreme deviation could be found.⁸⁶ An example might be the finding of lineage infidelity—that is, cells display markers specific for different lineages.¹⁰⁷ Doubly marked AML blast cells were shown indicating the simultaneous expression of TdT, characteristic for lymphoid cells and of monoclonal antibodies with myeloid specificity.¹⁰⁸ On the other hand, this concurrent expression of lineage specific markers of different axes might represent correlates of normal cell properties and thereby indicates that these leukaemic cells were frozen at a certain differentiation position characterised by multiple markers.⁸⁶

Conclusions

Determination of enzyme concentrations in leukaemic cells shows quantitative alterations of gene expression; by demonstrating the isoenzyme patterns, qualitative aspects of gene expression can be detected. Characteristic and distinct enzyme patterns exist which help to distinguish main groups and subsets of acute leukaemia by indicating cell lineage, differentiation stage, and cell function.

Characterisation of leukaemic cells on an immunological and enzymatic basis had led to a re-evaluation of traditional classification schemes of leukaemia and models of normal haematopoietic cell differentiation. An active assistance in the exploration of the pathobiology of leukaemia is provided by enzyme marker analysis, which has become

Table 5 Specific inhibition of enzymes

Target enzyme	Inhibitor	Original report	Application
Adenosine deaminase	2'-deoxycoformycin	Smyth <i>et al</i> ⁹⁰	In therapeutic but still experimental use; mostly applied to refractory cases
Purine nucleoside phosphorylase 5'-nucleotidase	8-aminoguanosine α , β -methylene adenosine diphosphate	Kazmers <i>et al</i> ¹⁰² Edwards <i>et al</i> ¹¹⁵	Studied in permanent leukaemia cell lines Still used only for the biochemical characterisation of the enzyme
Terminal deoxynucleotidyl transferase	6-anilinouracils (GW-17E, GW-18c)	McCaffrey <i>et al</i> ⁹⁶	In vitro using cell lines; in vivo in animal models

particularly productive since it has been included in the multivariate analysis, a multidisciplinary approach combining some otherwise independent research fields which is known as "multiple marker analysis."³

The concept that a correlation exists between the expression of marker by leukaemic cells and normal differentiation programmes, described as "maturation arrest"¹⁰⁴ of leukaemic cells, is thus far in line with the results seen in enzymatic studies. An abnormal amplification of enzyme quantities and qualitative changes in the arrangement of isoenzymes might be explained as an "abnormal, deranged expression of normal gene products."¹⁰⁶ It was proposed that the patterns of enzymic alterations in leukaemic cells represent a manifestation of the "reprogramming of gene expression."¹⁰⁰

In turn, understanding these alterations in the enzymology and biochemistry of leukaemic cells made it possible to identify possible sensitive key enzymes as targets for chemotherapy. Thus, several inhibitors acting on the enzymes adenosine deaminase, purine nucleoside phosphorylase, and TdT have the potential for serving as cytotoxic agents with considerable selectivity for cells with high amounts of the particular enzymes. This pharmacological manipulation would represent a specific, practical application of results of basic research. Enzyme markers have proved to be of diagnostic and potential therapeutic importance. Four of the seven described enzymes were measured quantitatively in functional assays of total enzyme activity. In addition to the quantitative enzymatic differences there might be structural changes in the enzymes expressed in alterations of isoenzymes, shifts of isoenzyme patterns, or other biochemically detectable atypicalities. Studies looking for such qualitative (iso) enzyme abnormalities should be performed in the context of multiple marker analysis giving much more new information and consequently a better understanding of the biological nature of this disease than would do an isolated investigation.

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