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Expression of a Monocyte-specific Esterase Isoenzyme in Cases of Acute Myeloid Leukemias

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Abstract—*The carboxylic esterase (E.C. 3.1.1.1) isoenzymes from cases of acute myeloid leukemias were separated by analytical isoelectric focusing on horizontal thin-layer gels. One isoenzyme consisting of one or two components (bands) could be completely and selectively inhibited by addition of 40 mM sodium fluoride (NaF) to the staining bath. The 105 cases were classified into the groups M1–M6 according to the FAB proposals. The NaF-sensitive isoenzyme was not detected in cases of FAB groups M1/2 (acute myeloblastic leukemia without or with maturation), group M3 (acute promyelocytic leukemia) or group M6 (erythroleukemia). Thirty-one out of 33 cases in the FAB group M4 (acute myelomonocytic leukemia) and 9/9 cases in FAB group M5 (acute monocytic leukemia) expressed the NaF-sensitive isoenzyme. The NaF-sensitive isoenzyme was found at different staining intensities; all M5 cases showed the isoenzyme at strong or very strong intensity, whereas most of the M4 cases displayed the isoenzyme at weak, medium or strong staining intensity. The data presented are further evidence that the presence of the NaF-sensitive esterase isoenzyme indicates monocytic involvement or differentiation in cases of myeloid leukemias. The easy and fast to perform method of isoelectric focusing can be used to distinguish the monocytic variants among the acute myeloid leukemias and can supplement the morphological analysis of these cases.*

INTRODUCTION

ENZYMATIC activity in leukemia cells can be identified in various ways: intracytoplasmic localization of enzymes by cytochemical stains [1], quantitative measurement of intracellular enzyme levels [2] and qualitative demonstration of isoenzymes [3]. The enzyme carboxylic or non-specific esterase (E.C. 3.1.1.1) has been studied extensively in normal and malignant cells [2,3].

Non-specific esterase cytochemistry has been used traditionally as a supplementary stain to Wright–Giemsa staining in the classification of leukemias. Intense, diffuse reactivity covering the entire cell which is sensitive to inhibition with sodium fluoride (NaF) is considered a marker for monocytoid cells [1]. These monocyte esterases have been identified by electron microscopy as ectoenzymes on the plasma membrane [4]. Weaker, granular reaction products that are fluoride resistant are detected in the cells of the granulopoietic series. A focal, discrete, intracellular membrane-bounded pattern of reactivity that is fluoride resistant has been described to be characteristic for T. lymphocytes, but has also been found in B lymphocytes [1].

An additional definition of the enzymology of the carboxylic esterases in hematopoietic cells was made possible by the separation of the enzymatic activity into its isoenzymes on the basis of electrophoretic mobility and isoelectric points [5]. Li *et al.* [6] were the first to show that defined populations of blood cells express characteristic esterase isoenzymes. These and other authors applied the method of disc electrophoresis in the analysis of normal [6, 7] and leukemia–lymphoma cells [6, 8, 9]. The technique of isoelectric focusing in vertical gel slabs enabled an even better resolution of isoenzymatic components and led to the detection of blood cell type characteristic or -associated isoenzyme profiles [10–13]. Isoelectric focusing on horizontal thin-layer gels allowed for a more easily performed and better separation of the isoenzymes yielding reproducible, clear isoenzyme patterns of the esterase isoenzymes from normal cells [14, 15], leukemia cells [16–19], lymphoma cells [20] and cultured, permanently established leukemia–lymphoma cell lines [21, 22].

In the study presented here we analyzed the carboxylic esterase isoenzyme profiles of 105 cases of acute myeloid leukemias with regard to the

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expession of a particular isoenzyme which can be completely and selectively inhibited by sodium fluoride. All cases have been classified into the categories M1–M6 on the basis of morphological–cytochemical criteria as defined by the French–American–British (FAB) co-operative group [23].

MATERIALS AND METHODS

Cells

Peripheral blood and/or bone marrow samples were obtained from patients with acute myeloid leukemias. Diagnoses were established on the basis of standard clinical, morphological, cytochemical and immunological criteria. Mononuclear cells were separated by Ficoll–Hypaque density gradient centrifugation. Only samples containing 90% or more blast cells were analyzed.

All cases were classified into the categories M1–M6 on the basis of the described criteria of the FAB-groups [23].

Preparation of enzyme solutions

Subsequent to the harvest of the mononuclear cell populations, cells were resuspended in a Tris–sucrose buffer, pH 7.4, at a concentration of 5×10^7 cells/ml [21]. Enzymes were extracted by three cycles of freezing and thawing. Enzymatic activity was solubilized by addition of 1% Triton × 100. After centrifugation, the cell-free supernatant was used for analytical isoelectric focusing. Aliquots of 'enzyme solution' referring to equal numbers of cells were applied on the gels.

Separation into isoenzymes

Analytical isoelectric focusing was performed on horizontal thin-layer gels using a LKB-Multiphor system (LKB, Bromma, Sweden). Gel matrix was composed of 4.8% (w/v) polyacrylamide, 12.5% (w/v) sucrose, 0.015% (w/v) ammoniumpersulfate/riboflavin, 0.1% (v/v) tetramethylethylenediamine and 2% (w/v) ampholyt of pH range 2–11 (Serva, Heidelberg, F.R.G.). Runs were performed for 1 hr at 5°C and 30 W constant power, with the voltage limited to 1400 V [21].

Staining of isoenzymes and enzyme inhibition

Isoenzymes were visualized directly on the gels using a solution of phosphate buffer (pH 7.2), alpha-naphthylacetate dissolved in acetone and Fast Blue RR. Gels were stained for 60 min at room temperature [21].

For enzyme inhibition, 40 mM NaF was added to the staining bath.

RESULTS

Classification of cases

The 105 cases studied were classified morphologically into main groups according to the FAB proposals by three hematologists–pathologists [23]. FAB groups M1 and M2 represented acute myeloblastic leukemia (AML) without or with maturation (M1/2, $n = 51$), group M3 represented acute promyelocytic leukemia (APL, $n = 7$), group M4 represented acute myelomonocytic leukemia (AMMoL, $n = 33$), group M5 represented acute monocytic leukemia (AMoL, $n = 9$) and group M6 represented erythroleukemia ($n = 5$) (Table 1).

Expression of sodium fluoride-sensitive isoenzymes in acute myeloid leukemias

In 40 out of 105 cases analyzed one or two bands were detected in the isoenzyme profile which could be completely and selectively inhibited by addition of NaF (Fig. 1), whereas all other bands were equally visualized in both staining baths with or without NaF.

None of the cases in FAB groups M1/2, M3 and M6 showed the NaF-sensitive bands; only cases of monocytoid leukemias (FAB M4 and M5) expressed these bands (Table 1). Thirty-one out of 33 cases of FAB group M4 and 9/9 cases of FAB group M5 were positive for this enzyme marker. The staining intensity of the NaF-sensitive isoenzyme varied between the positive cases. The relative intensity of the bands was judged as weak, medium, strong or very strong (Table 2). Most cases of FAB group M4 displayed the NaF-

Table 1. Expression of NaF*-sensitive esterase isoenzyme in acute myeloid leukemias categorized according to FAB criteria*

	FAB groups					
	M1/2 ($n = 51$)	M3 ($n = 7$)	M4 ($n = 33$)	M5 ($n = 9$)	M6 ($n = 5$)	Total ($n = 105$)
Cases negative for isoenzyme	51	7	2	0	5	65
Cases positive for isoenzyme	0	0	31	9	0	40

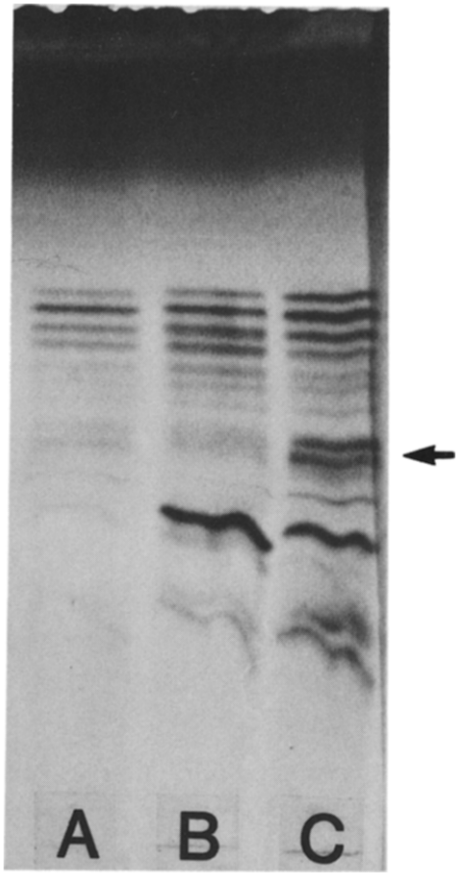

Fig. 1. Carboxylic esterase isoenzyme profiles of cases with acute myeloid leukemias separated by isoelectric focusing. (A) Acute myeloblastic leukemia, FAB M1. (B) Acute promyelocytic leukemia, FAB M3. (C) Acute myelomonocytic leukemia, FAB M4; arrow indicates two NaF*-sensitive isoenzyme bands of strong staining intensity. (Top cathode, bottom anode.)*

Table 2. Relative staining intensity of NaF*-sensitive esterase isoenzyme in cases of acute myelomonocytic (FAB M4) and monocytic (FAB M5) leukemia*

Staining intensity of isoenzyme	FAB groups M4 (n = 31)	M5 (n = 9)
Weak	10	0
Medium	9	0
Strong	9	5
Very strong	3	4

sensitive isoenzyme at weak, medium or strong staining intensity, whereas this isoenzyme was stained strong or very strong in all cases of FAB group M5.

DISCUSSION

Several studies have demonstrated that the NaF-sensitive carboxylic esterase isoenzyme is specific for monocytes. Other authors used the term 'lysosomal acid esterase (E.C. 3.1.1.6)' [24, 25]. However, the enzyme with the E.C. number 3.1.1.6 was named 'acetylesterase' (enzyme nomenclature recommended by the International Union of Biochemistry). Furthermore, it was shown that the esterase activity is localized on the outer plasma membrane of monocytes and not in lysosomes [4]. Gels stained at pHs 5.8 and 7.2 gave virtually identical isoenzyme profiles, certainly identical with regard to the NaF-sensitive isoenzyme [16, 21]. Therefore it was concluded that the isoenzymes described here and the 'acid esterase' mentioned belong to the group of carboxylesterases [18, 21].

Depending on the pH range of ampholyt used, 1–6 bands which were NaF-sensitive and with isoelectric points of pH 5.7–6.2 were detected in normal cells [14, 15, 18], leukemia cells [16, 26] and leukemia cell lines [21]. In studies using purified cell populations it was demonstrated that this isoenzyme is specific for monocytes [24, 27]. The same isoenzyme was observed in macrophages [25], in myeloid leukemia cell lines of monocytic origin [21, 28] and in monocytic leukemias [19, 26]. This isoenzyme, which was not expressed by the untreated cells, could be induced by the phorbolester 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in the leukemia cell line HL-60 during induction of differentiation [29, 30, Drexler *et al.*, manuscript submitted]. HL-60 cells which originated from a case of acute promyelocytic leukemia are triggered by TPA to differentiate into monocyte-/macrophage-like cells [29].

In our analysis of 105 cases of acute myeloid leukemias isoelectric focusing of carboxylic esterase revealed the presence of the NaF-sensitive isoenzyme only in the cases of FAB M4 and M5 morphology, i.e. with myelomonocytic or monocytic nature, but not in the non-monocytic leukemias of FAB groups M1/2, M3 and M6. These results are further evidence that the NaF-sensitive isoenzyme is an enzyme marker for cells of monocytic origin. The cases of FAB group M5 (AMoL) generally showed a stronger staining intensity of the focused bands than the cases of FAB group M4 (AMMoL); therefore it appears that the M5 blast cells are more differentiated along the monocytic cell lineage than the M4 blast cells as more mature cells usually express stronger isoenzyme bands [21]. We [Drexler *et al.*, manuscript submitted] and others [28] could demonstrate that the staining intensity of the monocyte-specific isoenzyme gradually increased during induction of differentiation in the monocytic leukemia cell lines ML-3, THP-1 and U-937, paralleling the progression of cellular maturation.

In the present and earlier studies [16, 21] we found that cells committed to the granulopoietic cell lineage without signs of monocytic nature also show esterase isoenzymes focused at about the same location in the gel (i.e. with the same or similar isoelectric points) as the NaF-sensitive isoenzyme; however, these 'granulopoietic esterase isoenzymes' were NaF-resistant at the concentration of NaF used. Therefore, in addition to the isoenzyme location, the NaF-sensitivity or NaF-resistance of the isoenzyme is of importance for the differentiation between cells associated with either the granulopoietic or monocytic cell lineage. However, staining of the isoenzyme in the presence of NaF at concentrations of 100 mM or higher led to a weaker staining intensity of most isoenzymes and eventually to complete inhibition of most isoenzymes [Drexler and Gaedicke, unpublished data].

There are limitations to a morphological and cytochemical subclassification of acute myeloid leukemias using the FAB classification. The overall concordance rate for subclassification into FAB M-groups by independent observers was in the range of 60–70% in several series [31–33]. The most common discrepancies in these studies were M1 vs M2, M1 vs M4 and M2 vs M4 classifications [31, 32]. The use of the monocyte esterase isoenzyme for the reliable detection of the monocytoid element in these cases could improve the reproducibility of the FAB system and diminish especially the M1/2 vs M4 classification errors.

The findings presented here and those of other investigators taken together demonstrate that the NaF-sensitive esterase isoenzyme is an enzymatic marker for monocytic involvement or differentiation. Since the isoelectric focusing system using several enzymes (carboxylic esterase, acid phos-

phatase, hexosaminidase and lactate dehydrogenase) is, on the one hand, an inexpensive technique and fast and easy to perform, and on the other makes a clear distinction between acute lymphoid and myeloid leukemias [19] and between myelocytic and monocytic variants, we are investigating its utility in the classification of acute leukemias as part of the multiple marker analysis. Multiple marker analysis [2, 3] combines the disciplines of morphology, cytochemistry, immunology, pathology and enzymology in order to establish a diagnosis which is as accurate as possible.

REFERENCES

1. Flandrin G, Daniel M-Th. Cytochemistry in the classification of leukemias. In: Catovsky D, ed. *The Leukemic Cell.* Edinburgh, Churchill-Livingstone, 1981, 29–48.
2. Drexler HG, Gaedicke G, Minowada J. Enzyme markers in acute leukemias: advances during the last decade. *JNCI* 1984, **72**, 1283–1298.
3. Drexler HG, Gaedicke G, Minowada J. Biochemical enzyme analysis in acute leukaemia. *J Clin Pathol* 1985, **38**, 117–127.
4. Bozdech MJ, Bainton DF. Identification of α-naphthyl butyrate esterase as a plasma membrane ectoenzyme of monocytes and a discrete intracellular membrane-bounded organelle in lymphocytes. *J Exp Med* 1981, **153**, 182–195.
5. Gaedicke G, Drexler HG. The use of enzyme marker analysis for subclassification of acute lymphocytic leukemia in childhood. *Leuk Res* **6**, 437–448.
6. Li CY, Lam W, Yam LT. Esterases in human leukocytes. *J Histochem Cytochem* 1973, **21**, 1–12.
7. Yourno J, Mastropaolo W. Nonspecific esterases of the formed elements: zymograms produced by the pH 9.5 polyacrylamide gel electrophoresis. *Blood* 1981, **58**, 939–946.
8. Young GW, Bittar ES. Analysis of tissue esterases from patients with Hodgkin's disease and other types of advanced cancer by isoelectric focusing in acrylamide gel. *Cancer Res* 1973, **33**, 2692–2700.
9. Kass L, Peters CL. Esterases in acute leukemias. *Am J Clin Pathol* 1978, **69**, 57–61.
10. Kass L, Munster D. Non-specific esterases in human blood cells: characterization by isoelectric focusing in polyacrylamide gel. *Exp Hematol* 1979, **7**, 535–540.
11. Kass L, Munster D. Nonspecific esterases in leukemic blasts. *Am J Clin Pathol* 1979, **72**, 611–613.
12. Yourno J, Burkart P, Mastropaolo W, Tartaglia A. Nonspecific esterase of B lymphocytes from a case of chronic lymphocytic leukemia and of normal T lymphocytes: similar constellations of isoenzymes. *Blood* 1982, **60**, 24–29.
13. Yourno J, Burkart P, Lizzi F, Tartaglia A. Enzymologic classification of acute leukemias: nonspecific esterase marker distinguish myeloid and lymphoid varieties. *Blood* 1982, **60**, 304–308.
14. Radzun HJ, Parwaresch MR, Kulenkampff C, Staudinger M, Stein H. Lysosomal acid esterase: activity and isoenzymes in separated normal human blood cells. *Blood* 1980, **55**, 891–897.
15. Radzun HJ, Parwaresch MR. Isoelectric focusing pattern of acid phosphatase and acid esterase in human blood cells, including thymocytes, T lymphocytes and B lymphocytes. *Exp Hematol* 1980, **8**, 737–741.
16. Drexler HG, Gaedicke G. Isoenzyme studies in human leukemia. II. Carboxylic esterase (E.C.3.1.1.1). *Leuk Res* 1983, **7**, 599–609.
17. Scott CS, Limbert HJ, Bynoe AG, Roberts BE. Cytochemical and electrophoretic characterisation of alpha naphthyl acetate esterases (ANAE) in acute myeloblastic leukemia. *Blut* 1984, **49**, 331–338.
18. Oertel J, Kastner M. Two-dimensional separation of α-naphthyl acetate esterases in human leucocytes and platelets. *Br J Haematol* 1984, **57**, 431–439.
19. Gaedicke G, Drexler HG. Leukemic cell differentiation in childhood leukemias. Analysis by enzyme markers. *Eur J Pediatr* 1984, **142**, 157–164.
20. Parwaresch MR, Staudinger M, Radzun HJ. Analysis of acid esterase activity and polymorphism as an aid for the subclassification of human low-grade malignant non-Hodgkin's lymphomas. *Cancer* 1982, **50**, 2383–2387.
21. Drexler HG, Gaedicke G, Minowada J. Isoenzyme studies in human leukemia–lymphoma cell lines. I. Carboxylic esterase. *Leuk Res* 1985, **9**, 209–229.
22. Sundstroem C, Radzun HJ, Parwaresch MR. Isozymes of acid esterase and acid phosphatase in permanent human hematopoietic cell lines. *JNCI* 1982, **69**, 1255–1260.
23. Bennett JM, Catovsky D, Daniel M-Th *et al.* Proposals for the classification of the acute leukaemias. *Br J Haematol* 1976, **33**, 451–458.
24. Radzun HJ, Parwaresch MR, Kreipe H. Monocytic origin of human alveolar macrophages. *J Histochem Cytochem* 1983, **31**, 318–324.
25. Parwaresch MR, Radzun HJ, Dommes M. Origin and homogeneity of peritoneal macrophages in human. *Am J Pathol* 1981, **102**, 209–218.
26. Scott CS, Linch DC, Bynoe AG *et al.* Electrophoretic and cytochemical characterization of

alpha-naphthyl acetate esterases in acute myeloid leukemia: relationships with membrane receptor and monocyte-specific antigen expression. *Blood* 1984, **63**, 579–587.
27. Radzun HJ, Parwaresch MR, Wittke JW. Polymorphism and antigenic specificity of monocytic acid esterase (E.C.3.1.1.6). *Cell Immunol* 1981, **63**, 400–408.
28. Radzun HJ, Parwaresch MR, Sundstroem C, Nilsson K, Eissner M. Monocytic origin of the human hematopoietic cell line U–937 and its convertibility to macrophages evidenced by isoenzyme mapping. *Int J Cancer* 1983, **31**, 181–186.
29. Parwaresch MR, Radzun HJ, Boedewadt S, Frendel A, Sundstroem C, Lennert K. Alternative myelomonocytic differentiation of HL–60 reflects dual prospective potency of promyelocytes in human. *Cell Immunol* 1984, **89**, 385–398.
30. Yourno J, Walsh J, Kornatowski G, O'Connor D, Kumar SA. Nonspecific esterase of leukemia cell lines: evidence for activation of myeloid-associated zymograms in HL–60 by phorbol esters. *Blood* 1984, **63**, 238–241.
31. Head DR, Savage RA, Cerezo L *et al.* Reproducibility of the French–American–British classification of acute leukemia: the Southwest Oncology Group Experience. *Am J Hematol* 1985, **18**, 47–57.
32. Sultan C, Deregnancourt J, Ko YW *et al.* Distribution of 250 cases of acute myeloid leukaemia (AML) according to the FAB classification and response to therapy. *Br J Haematol* 1981, **47**, 545–551.
33. Bennett JM, Begg CB. Eastern Cooperative Oncology Group Study of the cytochemistry of adult acute myeloid leukemia by correlation of subtypes with response and survival. *Cancer Res* 1981, **41**, 4833–4837.