Enzymologic Classification of Acute Leukemias: Nonspecific Esterase Markers Distinguish Myeloid and Lymphoid Varieties

By Joseph Yourno, Peter Burkart, Frank Lizzi, and Anthony Tartaglia

Nonspecific esterase zymograms of purified leukemic cells from a case of acute myelomonocytic leukemia (AMML) and from a case of acute non-B, non-T lymphocytic leukemia (ALL) were prepared as previously described. Leukemic cells were purified by gravity sedimentation with dextran (ALL) or without dextran (AMML) from the therapeutic leukapheresis preparations in citrate anticoagulant. Purified leukocytes were suspended in 3–5 vol of 20 mM n-morpholineethanesulfonic acid (MES), pH 6.4, containing 20% glycerol for storage at −20°C.

Smears of buffy preparations, Romanowsky stains, and nonspecific esterase cytochemistries were made as previously described. Naphthol AS-D chloracetate (NASDCLA) esterase cytochemistry was performed similarly. Peroxidase (Px) cytochemistry was according to the method of Kaplow. Procedures for extraction of nonspecific esterases have been previously described, as has the procedure for spectrophotometric assay of nonspecific esterase. Isoolectric focusing of esterases was performed with an LKB Multiphor IEF unit (LKB Instruments, Hicksville, N.Y.). Freshly prepared 5% polyacrylamide gels containing LKB ampholites (pH range 3.5–9.5) were used. Aliquots (20–25 μl) of leukocyte extract containing 3.0 ± 0.5 mU αNB activity were pipetted onto Miracloth strips positioned either at midgel or toward the alkaline extremity. Runs generally required 2–2.5 hr to reach the end-point of 1200–1400 V at 20 mA. Gels were stained directly with αNA or αNB substrate, prepared as previously described. The linear pH gradient established by isoelectric focusing was confirmed by pH determination of extracts of gel segments and by running protein standards (Pharmacia Fine Chemicals, Piscataway, N.J.).

RESULTS

Cytomorphological and cytochemical analyses of buffy preparations used showed that leukemic cells accounted for almost 99% of cells in each case. Wright's stained smears of the AMML preparation contained 97% blasts and 2% leukemic monocytes with pronounced nuclear-cytoplasmic dissociation (Fig. 1A). From 45% to 75% of cells showed moderate to intense α-naphthyl acetate (αNA) esterase and α-naphthyl butyrate (αNB) esterase reactivity in a scattered granular pattern that was sensitive to 40 mM

CYTOCHEMISTRY has gained wide acceptance as a supplement to Romanowsky staining in the classification of acute leukemias. Despite the increased resolving power lent by supplemental cytochemistries, a significant fraction of cases gives fragmentary or equivocal cytochemical profiles that are of uncertain diagnostic value. We have sought to develop more objective enzymologic criteria for classification of acute leukemias through zymogram analysis of nonspecific esterases. We demonstrate that isoelectric focusing profiles clearly differentiate leukemic cells from a case of acute myelomonocytic leukemia (AMML) and from a case of non-B, non-T acute lymphocytic leukemia (ALL).

MATERIALS AND METHODS

An AMML leukapheresis preparation was obtained from a 57-yr-old white woman who underwent emergency pheresis on admission white count was 282 × 10⁹/liter, >95% leukemic cells). The bone marrow was hypercellular with 90% leukemic blasts and granulocyte zymograms. On the other hand, nonspecific esterase of ALL showed a striking departure from the zymogram pattern of control B lymphocytes and T lymphocytes. An intense reactivity with a very low isoelectric point accounted for most of the ALL nonspecific esterase activity. No corresponding reactivity or relatively small amounts thereof were seen in other zymograms. Conversely, few of the isoenzymes that were prominent in zymograms of control lymphocytes were apparent above trace levels in ALL zymograms. Thus, zymogram analysis of nonspecific esterases clearly differentiated the myeloid leukemia from the lymphoid leukemia and provided a potential marker for each. The AMML cells appeared well enough differentiated with respect to nonspecific esterases as to be similar to mature cells of like lineage. It is plausible that the ALL cells, however, were arrested at an earlier stage of esterase expression as reflected by the associated atypical species.

From the Laboratory Medicine Institute, Center for Laboratories and Research, New York State Department of Health, and the Division of Hematology, St. Peter's Hospital, Albany, N.Y. Submitted September 21, 1981; accepted March 10, 1982. Address reprint requests to Joseph Yourno, Laboratory Medicine Institute, Center for Laboratories and Research, New York State Department of Health, Albany, N.Y. 12201.

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*The term "myeloid" as used here includes both granulocytic and monocytic lineages.
Fig. 1. Cytomorphology and nonspecific esterase cytochemistry of purified leukemic cells (before red cell lysis). (A) AMML, Wright’s stain (200×). Leukemic blasts with high to moderate N/C ratio, with smaller forms superficially resembling lymphocytes. Few maturing monocytoid cells or granulocytic cells are evident. (B) AMML, αNB stain (160×). Cells show scattered granular activity ranging from strong or intense (21%) to weak or negative (49%). (C) AMML, αNB stain with 40 mM sodium fluoride (160×). Strong to intense activity is obliterated, most cells show undetectable to weak scattered granular activity, occasional cells moderate activity. (D) ALL, Wright’s stain (160×). Leukemic blasts, prolymphocytes, and lymphocytes, most with a high N/C ratio and thin rim of cytoplasm. (E) ALL, αNB stain. Virtually all cells show weak to moderate granular reactivity, predominantly in a scattered granular pattern. Occasional cells show a more focal reactivity. (F) ALL, αNB with 40 mM sodium fluoride (160×). Partial inhibition of nonspecific esterase with cells demonstrating substantially weaker activity.
Zymogram analysis of nonspecific esterases prepared by isoelectric focusing clearly differentiated leukemic cells of a case of AMML from those of a case of ALL and provided a potential marker for each. The benchmark classification of these acute leukemias was based on standard cytomorphological, cytochemical, and cytoimmunologic criteria.

As we expected from their monocytoid-granulocytic cytochemical behavior, zymograms of AMML cells closely matched those of control monocytes and granulocytes. The key feature was a set of fluoride-sensitive myeloid enzymes in AMML, monocyte, and granulocyte zymograms, which were undetectable or visualized at trace levels only in lymphocyte and ALL zymograms. The set of myeloid isoforms detected here by isoelectric focusing matches the αNA esterases originally described in monocytes by Radzun et al. Most myeloid leukemias demonstrate substantial nonspecific esterase cytochemically. It is plausible that a myeloid nonspecific esterase zymogram is characteristic of the entire spectrum of “esterase-positive” myeloid leukemias from pure acute myeloblastic (AML) to pure acute monocytic types (AMOL). The level of myeloid isoforms may vary as a function of degree and direction of cell differentiation (low in myeloblastic to high in monocytic). The relative fluoride resistance of granulocytic cytochemical nonspecific esterase presumably reflects the greater proportion of fluoride-resistant isoforms in these cells, which have only modest levels of the myeloid species. In the minority of cases of myeloid leukemia, which show weak to negative esterase reactivity, the myeloid esterases may be retained or may be lost.

The literature on nonspecific esterase of ALL cells is conflicting. Reports on substantial series of cases differ widely on the extent and pattern of cytochemical reactivity in ALL, as well as on the relationship of cytochemical findings to immunologic subtypes. The nonspecific esterase cytochemistry of ALL cells in this study was similar to that of mature lymphocytes (moderate αNA and αNB reactivity partially resistant to sodium fluoride inhibitor). Yet, the ALL zymograms bore little resemblance to those of (CLL) B-lymphocyte or normal T-lymphocyte controls or of any other type of cell studied. The bulk of the nonspecific

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Fig. 2  (A) Zymograms of nonspecific esterase of leukemic cells, isoelectric focusing, pH 3.5 (bottom) to 8.5 (top), simultaneously run on one slab. Each specimen contained $3.0 \pm 0.5$ mU αNB esterase. Left panel, αNB, right panel αNA. Left to right: Mono, monocyte control; AMML; BL, (CLL) B-lymphocyte control; ALL. Specimens were applied in the area of final pH 7.5–8.0 where some precipitate can be seen. Application at this point enhanced visualization of the ALL-associated species with a low isoelectric point. (B) Zymograms of nonspecific esterase of leukemic cells, isoelectric focusing, pH 3.5 (bottom)–8.5 (top), simultaneously run on one slab. Each specimen contained $3.0 \pm 0.5$ mU αNB esterase. Left panel, αNB substrate; right panel, αNA substrate. From left to right: Mono, control monocytes; Gran, control granulocytes; AMML; BL, control (CLL) B lymphocytes; TL, control T lymphocytes; ALL. Specimens were applied in the area of final pH 7.0–7.5, where some precipitate can be seen. Application at this point enhanced visualization of the myeloid species, with a neutral or moderately acidic isoelectric point. The “stepladder” effect of weak horizontal bands across the gels is caused by nonenzymatic binding of coupler dye by focused ampholines. The characteristic feature of AMML, monocyte, and granulocyte zymograms is a relatively prominent, closed spaced series of acetyl-butyryl esterase isoenzymes with an isoelectric point around pH 6-6.5. Since this series is especially powerful in monocytes and accounts for most of the nonspecific esterase activity, other esterase species are weakly visualized in the monocyte zymogram. The levels of this series are most modest in granulocytes and in the AMML cells, so that other esterase species are visualized as well in their zymograms at equivalent enzyme loading. The ALL zymogram shows an intense acetyl-butyryl esterase with an isoelectric point around pH 4. This species is undetectable or present in relatively small amounts in other zymograms, including lymphocyte controls. Lymphocyte controls show a widely spaced series of isoenzymes from pH 4.5 to 8. Relatively low levels of other isoenzymes are detectable in the ALL zymograms.
esterase extracted from the ALL cells seems ascribable to an intensely reactive species with a very low isoelectric point.

The results suggest that the AMML cells were highly differentiated with respect to lysosomal nonspecific esterases so as to resemble cells of like lineage. On the other hand, the data for the cells of this case of ALL are consistent with an arrest at an earlier state of differentiation, as reflected by the associated, atypical, nonspecific esterase species. The nature of this species, possibly a precursor or oncofetal form, remains to be determined. The general association of these potential markers with the various leukemias also remains to be determined. Certainly ALL is a heterogeneous class of leukemias which may produce a multiplicity of associated esterase zymograms. These data hold promise for a new index of leukocyte differentiation and a more objective enzymologic classification of acute leukemias. We are attempting to extend this analysis of leukemic bone marrow specimens and peripheral bloods.

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