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β-Hexosaminidase Isoenzyme I: An Early Marker of Hematopoietic Malignancy *

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A. Introduction

The analysis of various enzymes has been found to distinguish immunologic subsets of human leukemias. This is especially true for terminal deoxynucleotidyl transferase (TdT). This enzyme is present in 90% of all cases with common ALL, pre B-ALL, T-ALL, and it is negative in B-ALL. Some 5%–15% of AML are also positive for TdT. Interestingly, acute undifferentiated leukemias (HLA-DR positive, cALL-A negative) have been found to be TdT positive in 40%–60% of cases. This nuclear enzyme thus seems to be a very early marker of lymphohematopoietic development. The lysosomal enzyme β-hexosaminidase (*N*-acetyl-β-D-glucosaminidase) can be separated into two major forms: an isoenzyme A, which is constituted by two α and two β subunits, and in isoenzyme B, which consists of four β subunits. In cALL cells, a third isoenzyme was first observed by Ellis et al., eluted from an ion exchange chromatographic resin in an intermediate position between the A and B forms [1]. This intermediate isoenzyme I seems to consist of β subunits. Hexosaminidase C is a fourth isoenzyme with a more acidic isoelectric point than hexosaminidase A. In the study presented here, hexosaminidase isoenzymes were investigated as part of multiple marker analysis in various leukemia subtypes.

B. Methods

Leukemia cells were isolated from fresh bone marrow aspirates of leukemic patients, and separated by Ficoll and Percoll step gradients or by counterflow elutriation. Between 10^7 and 10^8 cells were used for the enzymatic tests. A crude extract was prepared by sonic disruption of the cells, isolation of the lysosomal fraction, and solubilization with a nonionic detergent. Activity was measured with the α-phenyl method, and protein concentration according to the Lowry procedure [2].

The enzyme was enriched either by phenyl–sepharose chromatography or by a Con-A–sepharose column prior to DEAE ion exchange chromatography. This was done with a LKB high performance system, using a linear NaCl gradient from 0 to 0.5 *M* NaCl. Crude extracts were used for separating the enzymatic activity into its isoenzymes by analytic isoelectric focusing or disk electrophoresis [2].

C. Results

1. In leukemic specimens, isoelectric focusing clearly demonstrates an anodic shift of hexosaminidase A. The same phenomenon is seen in DEAE ion exchange chromatography.

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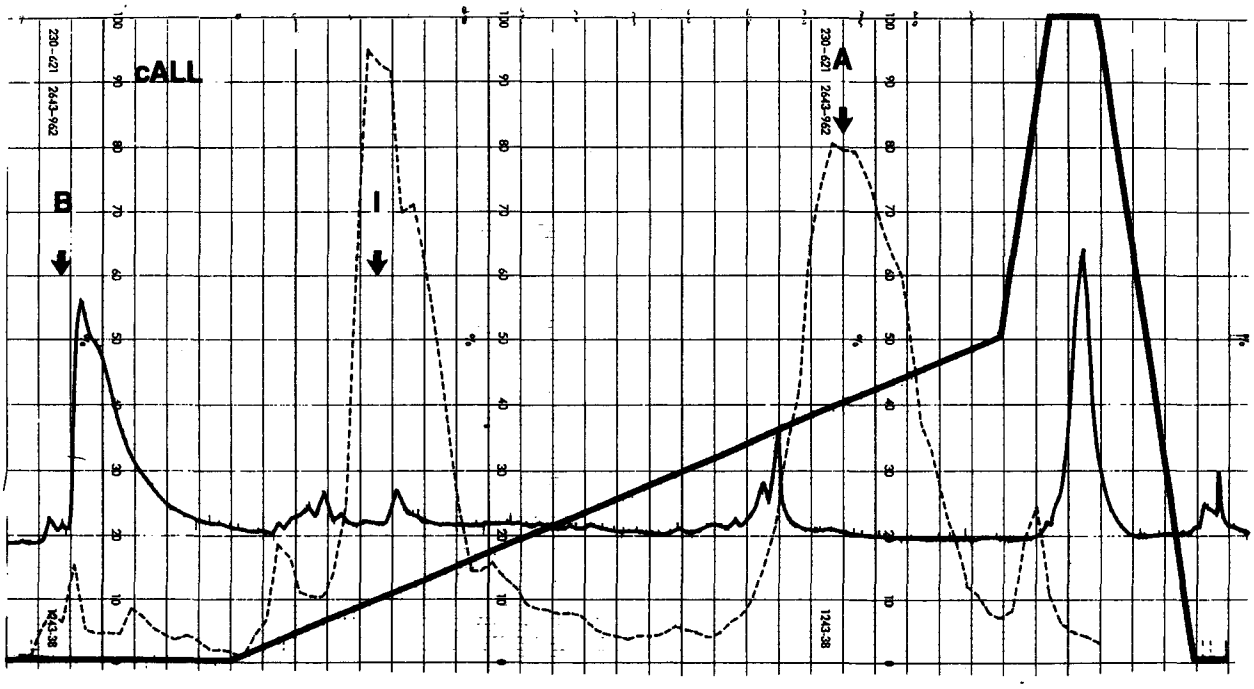

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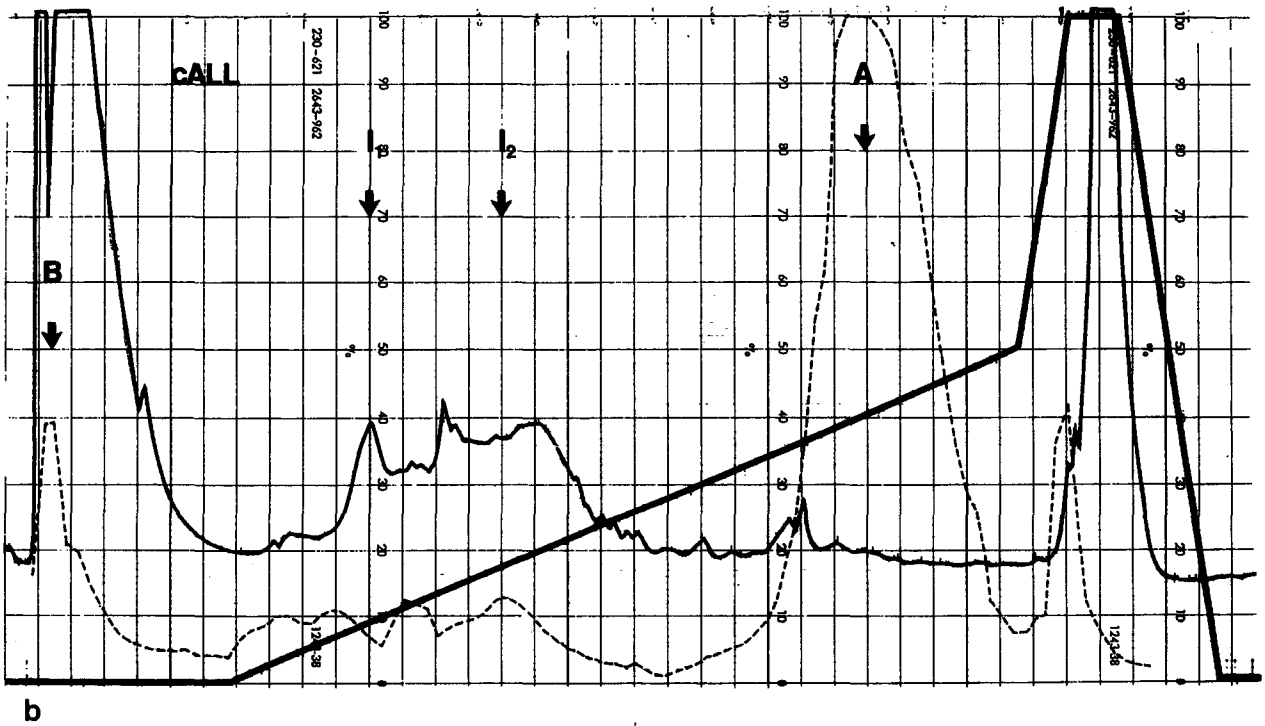

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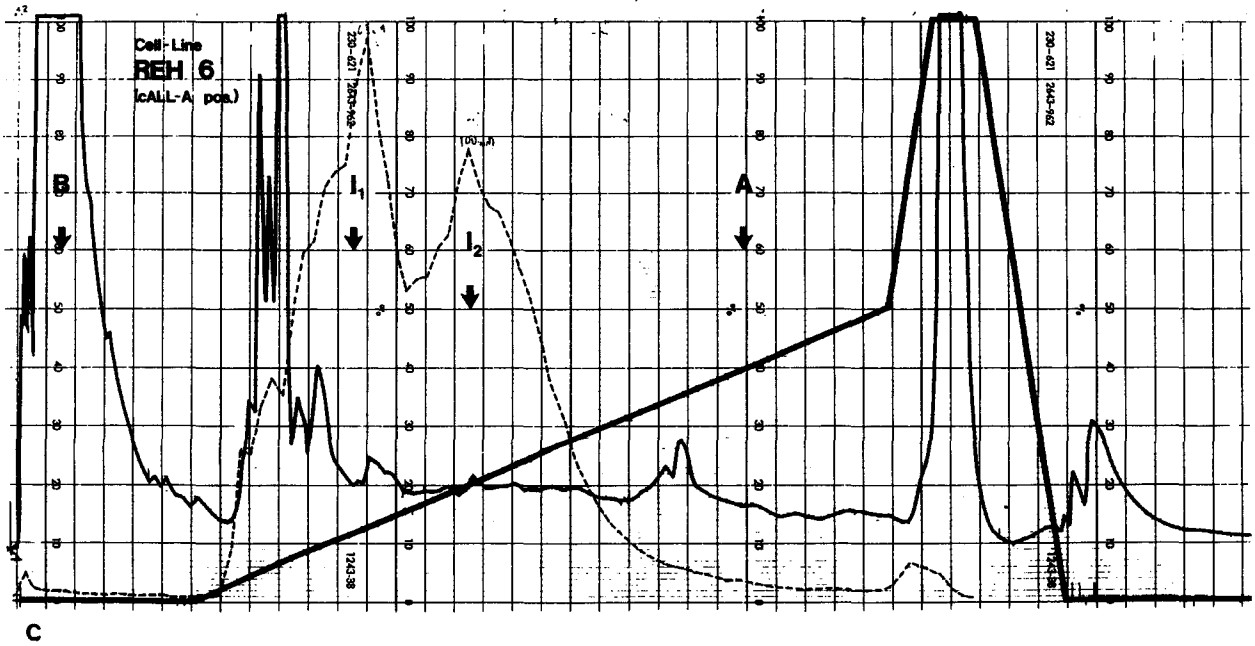

c

Fig. 1 a–c. Ion exchange chromatographic patterns of hexosaminidase isoenzymes. *Thick full line* NaCl gradient; *thin full line* OD tracing; *broken line* hexosaminidase activity. *Arrows* indicate the position of isoenzymes A, B, and I. In the cell line REH 6, tow peaks with intermediate position are eluted from the DEAE column, indicating that isoenzyme I occurs in at least two variant forms

2. The investigation of 15 cases of acute childhood leukemias using a comparative analysis of multiple surface markers with hexosaminidase isoenzymes, showed that cALL could be further subdivided. Of 15 cases, 3 had no hexosaminidase activity; 10 cases demonstrated isoenzyme A, B, and I (Fig. 1 a), 2 cases isoenzymes A and B only (Fig. 1 b). Acute lymphocytic leukemias had imbalanced synthesis of A and B forms with a decrease of the hexosaminidase isoenzyme B. The cALL-positive celle line REH subclone 6 had two enzymatic activities in the intermediate region, and no A or B isoenzyme (Fig. 1 c). T cell leukemias (13 cases) had no isoenzyme I. Acute myeloblastic leukemias (8 cases) either had isoenzymes A and B only, or A, B and I. However, the ratio of A : B isoenzyme activity was balanced as is usually seen in normal granulocytes. Only one myeloblastic leukemia (M_1) had hexosaminidase A activity only.

All eight cases of AUL (HLA-DR positive, cALL-A negative, TdT±) had very low or absent enzymatic activity in the isoenzmye B region. To date, half of the cases investigated have had high levels of isoenzyme I and low levels of hexosaminidase A.

D. Discussion

Most of the surface and enzyme markers clearly demonstrate that leukemia cells correspond to a certain developmental stage of lymphohematopoiesis. This is called cell lineage fidelity [3]. As far as hexosaminidase isoenzyme I is concerned, it remains unknown if this isoenzyme occurs in normal hematopoietic precursor cells. It is well recognized that glycosphingolipids are the natural substrates for the hexosaminidases [4]. For hexosaminidase I this remains unknown. If it is assumed that hexosaminidase I activity represents elements of hexosaminidase B, it is possible to postulate that in leukemia cells there is lack of a factor necessary for the transformation of isoenzyme I to the B form. This suggestion is further supported by the observation of a shift from hexosaminidase I to hexosaminidase B when differentiation is induced in human leukemia cell lines by conditioned media or TPA (Drexler et al., this volume).

Hexosaminidase isoenzymes have been under investigation for a number of years in human leukemias and it has been observed that a shift to the anode occurs in hexosaminidase A, if the isoenzymes are separated by electrophoretic techniques [2, 5, 6]. This suggests that a posttranslational modification of this isoenzyme does not take place in leukemia cells. From our observations, one may conclude that hexosaminidase isoenzymes offer the opportunity of investigating the regulation of normal gene products in human leukemia cells.

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