

# Acid DNase Activities in Peripheral, Mononuclear Blood Cells: A Possible Parameter to Detect Proliferating Cell Populations

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After electrophoresis in DNA-containing polyacrylamide gels, two acid DNase activities can be detected in peripheral, mononuclear cells of the human blood. One of these acid DNase activities correlates with cell proliferation; its isoelectrical point is at pI 7.4. By means of this DNase activity, a quantity of less than 1% leukemic cells can be detected. The increased acid DNase activity can indicate the proliferation of malignant cell populations and possibly the proliferation of cell populations during immunological reactions.

## Introduction

Proliferating and non-proliferating cells differ in their DNase activities. Cells with a high proliferating rate show more DNase activity than cells with a low proliferating rate [1]. Cells taken from fetal tissues have a higher DNase activity than cells from the corresponding tissues of an adult animal [2]. Adding DNases to fetal tissues is detrimental to the degree of cell differentiation [3, 4]. DNase I stimulates the proliferation of protozoa and of fertilized eggs from sea-urchins [5].

Four different DNase activities can be detected in human lymphocytes [6]. The acid DNase activities in human lymphocytes correspond to the DNase described by Slor [7] and Stern [8]; this DNase activity increases in parallel with DNA synthesis. The activity of one of the two acid DNases in PHA-stimulated lymphocytes increases parallel to the [<sup>3</sup>H]thymidine incorporation [9]. Compared to the corresponding non-malignant and non-proliferating cells, the activity of one of the two acid DNases is enhanced in human leukemic cells and mouse lymphoblasts (L 5178 Y cells) [10]. In the mouse the activity of acid spleen DNase increases in comparison to proliferation of cells after antigen injections [11].

*Abbreviations:* PHA, phytohemagglutinin; FACS, fluorescence analysis cell scanner; ALL, acute lymphatic leukemia.

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This study examined if proliferating cell populations can be detected by determining acid DNase activities in suspensions with proliferating and non-proliferating cells, and if the determination of acid DNase activities is a parameter for cell proliferation. The method may be useful, if FACS analysis is not available.

## Materials and Methods

### Chemicals

The sources were as follows: Ficoll, Biochrom, Berlin (F.R.G.), penicillin and streptomycin, Chemie Grünenthal, Stolberg (F.R.G.), phytohemagglutinin (PHA), Biochrom, Berlin (F.R.G.), TC medium 199, Flow, Bonn (F.R.G.), [<sup>3</sup>H]thymidine, The Radiochemical Center, Amersham (U.K.), galloctanine, Fluka, Buchs (Switzerland), potassium chrom(III)sulfate, Merck, Darmstadt (F.R.G.), ampholine pH 3.5–10 and pH 9–11, LKB, Bromma (Sweden), pH marker proteins and Coomassie Blue, Serva, Heidelberg (F.R.G.); all other chemicals were from Merck, Darmstadt (F.R.G.) and Serva, Heidelberg (F.R.G.).

### Isolation and stimulation of mononuclear cells

The lymphocytes and leukemic cells were isolated in a density gradient [12]. Aliquots were washed and the pellets were frozen at –70 °C until enzyme examination. Other cells were cultured or stimulated with PHA [13]; culture medium: 80% TC medium 199, 15% fetal calf serum, 5% penicillin-streptomycin solution;  $1 \times 10^6$  cells/ml culture medium; time of cultivation or stimulation: 72 h, 37 °C, 5% CO<sub>2</sub>, water-saturated atmosphere. The cultivated



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or stimulated cells were washed, and the pellets were kept at  $-70^{\circ}\text{C}$  until examination. Cells were stained with May + Grünwald/Giemsa solution for blast cell determination. For measuring [ $^3\text{H}$ ]thymidine incorporation  $2.5 \times 10^5$  cells/ml culture volume (2 ml cultures) were labelled with [ $^3\text{H}$ ]thymidine ( $3.7 \times 10^4$  Bq/ml cell suspension) for 4 h. The cells were precipitated with 10% trichloroacetic acid, and the washed precipitate was dissolved in 0.5 M NaOH. The incorporated radioactivity was measured. Friend LC/F cells and L 5178 Y cells were taken from a permanent cell culture.

#### *Determination of DNase activity after electrophoresis*

The determination of DNase activity was performed using the *in situ* detection of DNases in DNA-containing polyacrylamide gels following electrophoretic separation [6]. Three million cells were suspended in 30% sucrose in spacer gel buffer and broken by triple freezing and thawing. A final sample volume of the supernatant corresponding to an equivalent of  $1.6 \times 10^5$  cells was applied per gel. We used a 5% acrylamide spacer gel, pH 6.7, 0.176 M Tris- $\text{H}_3\text{PO}_4$ , and a 13.4% acrylamide separation gel, pH 8.8, 0.177 M Tris- $\text{H}_2\text{SO}_4$ . The electrophoresis was performed at 700  $\mu\text{A}$ /gel for 80 min with the anode at the bottom. The DNA concentration in the small pore gel was 0.3 mg/ml. After electrophoresis the gels were incubated for 4 h at  $37^{\circ}\text{C}$  in 0.1 M Na-acetate, pH 5.0 + 5 mM EDTA. The gels were stained with galloxyanine-potassium chrom(III)sulfate (0.15% galloxyanine, 5% potassium chrom(III)sulfate) for 12 h. After destaining with water the optical density of the gels was recorded by a densitometer. Unstained gel regions represent places of hydrolyzed DNA and zones of DNase activity. Mixtures of fresh isolated lymphocytes and leukemic cells were prepared direct before electrophoresis.

#### *Determination of DNase activity after isoelectric focusing*

The isoelectric focusing of the samples was performed in the microsystem with polyacrylamide gels [14]: 7.5% acrylamide, 10% sucrose, and 4% ampholine (mixture of pH 3.5–10 and pH 9–11); length of the gels: 5 cm; diameter: 2 mm; duration of focusing: 4 h; anode: 0.01 M  $\text{H}_3\text{PO}_4$ ; cathode:

0.02 M NaOH; initial voltage: 100 V; final voltage: 500 V. Per gel a sample volume corresponding to an equivalent of  $2.5 \times 10^5$  cells was separated. After isoelectric focusing, the gels were cut into slices and the DNase activity was determined in the individual slices. DNase assay for each slice [15]: 300  $\mu\text{l}$  0.2 mg/ml DNA in 0.1 M Na-acetate, pH 5.0 + 5 mM EDTA. The incubation was performed at  $37^{\circ}\text{C}$  for 4 h. Thereafter, 300  $\mu\text{l}$  of ice-cold 15% perchloric acid were added. The samples were cooled for 20 min at  $0^{\circ}\text{C}$ . The precipitate was removed from the solution by centrifugation and the extinction of the supernatant was measured at 260 nm. The extinction of each slice of a parallel gel with an incubating time of 0 min was subtracted as background. For pH determination each slice of a parallel gel was eluted in 300  $\mu\text{l}$  10 mM NaCl. The pH gradient in addition was checked with pH marker proteins.

#### *Statistical analysis*

For statistical analysis t-test was used. Ten healthy persons and six patients with ALL were examined.

## **Results**

After electrophoresis in DNA-containing polyacrylamide gels, two acid DNase activities (DNase activity A and DNase activity B, Fig. 1) can be demonstrated in human lymphocytes. Freshly isolated lymphocytes and cultivated lymphocytes after a cultivation time of 72 h do not show any difference in their acid DNase activities. In PHA-stimulated lymphocytes after 72 h, however, an increase of activity band B can be detected. The increase of activity correlates with blast cell formation and [ $^3\text{H}$ ]thymidine incorporation (Fig. 1).

An increased acid DNase activity band B is also identified in leukemic cells from ALL patients, in cultivated leukemic cells (friend LC/F), and in cultivated lymphoma cells (L 5178 Y) (Fig. 2).

In fresh prepared mixtures of leukemic cells from ALL patients and lymphocytes from healthy donors the acid DNase activity band B correlates with the proportion of leukemic cells in the cell suspension. Determining acid DNase activity band B, less than 1% of leukemic cells can be detected in a suspension of mononuclear cells of the blood (Fig. 3). Even a proportion of 0.3% of leu-

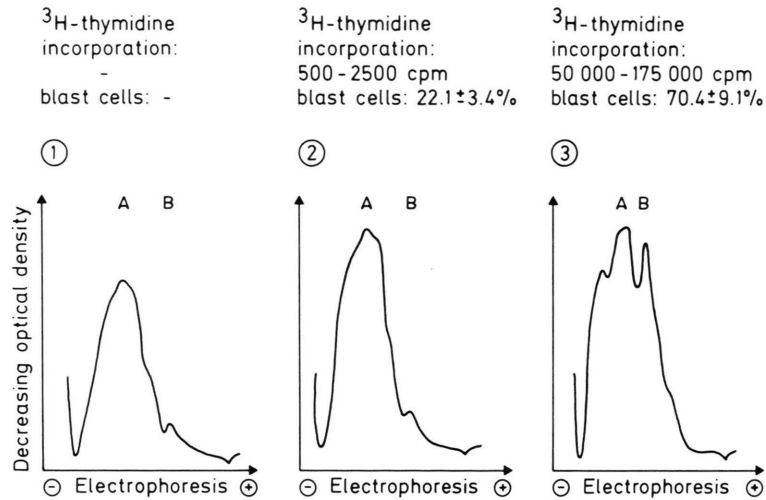


Fig. 1. Densitometer tracings of DNA-containing polyacrylamide gels after electrophoresis of freeze-thawed lymphocytes, cultivated lymphocytes, and PHA-stimulated lymphocytes, representative samples are shown, details see Methods. 1. Acid DNase activities A and B in lymphocytes; 2. acid DNase activities A and B in cultivated lymphocytes; 3. acid DNase activities A and B in PHA-stimulated lymphocytes.

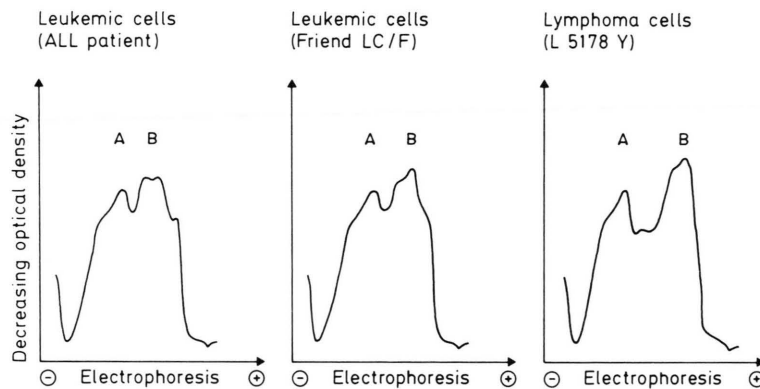


Fig. 2. Densitometer tracings of DNA-containing polyacrylamide gels after electrophoresis of freeze-thawed leukemic cells (ALL), Friend LC/L cells, and L 5178 Y cells, representative samples are shown.

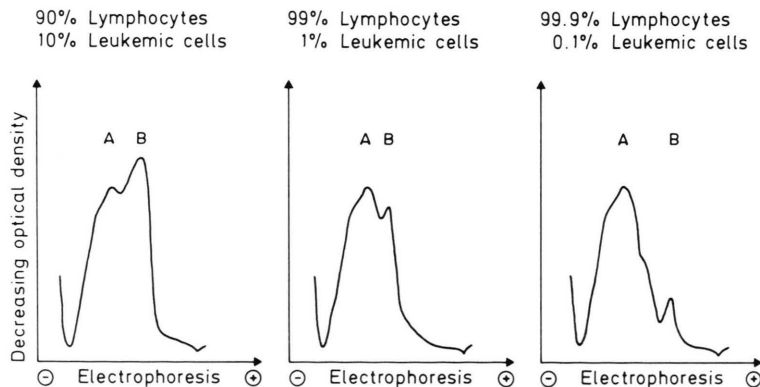


Fig. 3. Densitometer tracings of DNA-containing polyacrylamide gels after electrophoresis of freeze-thawed mixtures of lymphocytes (healthy persons) and leukemic cells (ALL patients), representative samples are shown, details see Methods.

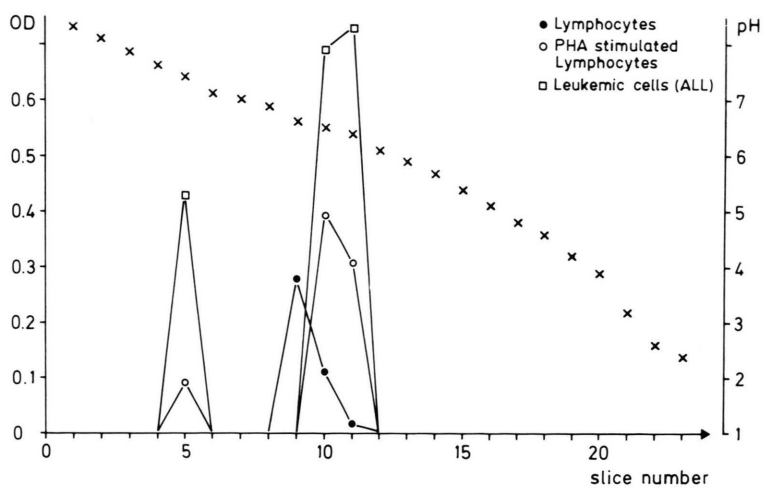


Fig. 4. Acid DNase activities in lymphocytes, PHA-stimulated lymphocytes, and leukemic cells (ALL) after isoelectric focusing in polyacrylamide gels, representative samples are shown, details see Methods. Abscissa: gel slice; ordinate: extinction of the acid-soluble supernatant of the DNase assay at 260 nm after background correction, pH of gel slices.

kemic cells shows differences in the activity of acid DNase activity band B in lymphocytes from healthy donors (Table I).

The isoelectric point of acid DNase activity is found between pH 6.5 and pH 6.6 in lymphocytes and between pH 6.4 and pH 6.5 as well as pH 7.4 in PHA-stimulated lymphocytes and in leukemic cells from ALL patients (Fig. 4). PHA-stimulated lymphocytes show a smaller acid DNase activity with pI 7.4 than leukemic cells. The DNase activity with pI 7.4 was eluted from the slices and then determined by electrophoresis in DNA-containing

polyacrylamide gels. DNase activity with pI 7.4 is in the electrophoretic running identical with acid DNase activity band B (Fig. 1).

Discussion

In human lymphocytes, two acid DNase activities (activity A and activity B) can be detected after electrophoresis. Both acid DNase activities are endonucleases originating from the nucleus [6]. Activity B differs from DNase II from pig spleen [6]. In stimulated lymphocytes, activity B correlates with blast cell formation and DNA synthesis. In resting cells (non-stimulated lymphocytes) activity B is low and in proliferating cells (stimulated lymphocytes) it is high according to proliferation. Acid DNase activities are probably important in DNA synthesis [9].

Presumably, the isoelectric point of acid DNase activity B is at pI 7.4, because the electrophoretic mobility is identical in both examinations. This activity cannot be demonstrated in resting lymphocytes after isoelectric focusing. At the same time, activity B is low, when the acid DNase with pI 7.4 is missing. However, in leukemic cells and in PHA-stimulated lymphocytes there exist a high proliferation and the acid DNase activity with pI 7.4 is detectable after isoelectric focusing. In these cases a high acid DNase activity B can also be observed after electrophoresis. In human lymphoblastoid cells Lambert [16] describes DNases with pI 6.6 and pI 7.6.

Table I. Acid DNase activity B in mixtures of leukemic cells from ALL patients and of lymphocytes from healthy donors. The amplitude of densitometer tracings of the activity in DNA-containing polyacrylamide gels (compare Fig. 1, 2, 3) was measured. The experiments were repeated for six times.

Lymphocytes [%]	Leukemic cells [%]	Amplitude of activity [mm] <sub>1/2</sub>
0.0	100.0	86 ± 3
90.0	10.0	74 ± 5
95.0	5.0	69 ± 6
99.0	1.0	58 ± 7
99.5	0.5	43 ± 6
99.7	0.3	35 ± 6
99.9	0.1	22 ± 7
100.0	0.0	14 ± 5

0.1% leukemic cells vs. 0.0% leukemic cells: not significant.  
0.3% leukemic cells vs. 0.0% leukemic cells: significant (p < 0.05).

After mixing resting and proliferating cells, one can determine the portion of proliferating cells in a cell suspension by using acid DNase activity B. In a suspension of lymphocytes a proportion of less than 1% of leukemic cells can clearly be identified. The method even works at a proportion of 0.3% of leukemic cells. Thus, the determination of activities of acid DNase activity B might be used for the control of cell proliferation in the blood. The determination of the activities can complement the microscopic evaluation of the blood. In addition, the method is useful parallel to FACS analysis or if a flow cytometer is not available.

The determination of acid DNase activity B can give two kinds of informations: 1. the proportion of proliferating, malignant cells in peripheral, mononuclear cells of the blood, and 2. the proportion of proliferating, non-malignant cells in peripheral, mononuclear cells of the blood.

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