Lysosomal Acid Esterase: Activity and Isoenzymes in Separated Normal Human Blood Cells


The striking difference in the cytochemical pattern of acid esterase between blood monocytes and T lymphocytes initiated the present study to determine whether or not the cytochemical difference is related to a cell-specific polymorphism of the isoenzymes. Enzyme assays and isoelectric focusing were performed using detergent-treated lysosomes from viable monocytes, granulocytes, T lymphocytes, platelets, and erythrocytes isolated from peripheral blood. B lymphocytes were separated from tonsils. Thymocytes were obtained from thymus glands excised during cardiac surgery. Except for monocytes, all cell suspensions showed a purity of more than 98%. The mean enzyme activity in monocytes amounted to 39 mU/10^7 cells. This value was 7 times higher than the activity level of lymphocytes, which showed values of 5.5 mU for 10^7 B lymphocytes, 5.3 mU for 10^7 T lymphocytes, and 8.1 mU for 10^7 thymocytes. Granulocytes exhibited the lowest enzyme activity. The isoelectric focusing pattern of monocytes disclosed 4 isoenzymes, with the anodic one accounting for more than 85% of the total activity. T lymphocytes had 13-16 bands distributed in 3 complexes between pH 7.9 and 4.5. Thymocytes displayed a similar pattern, with only 11 bands. B lymphocytes showed 7 isoenzymes between pH 6.4 and 5.5. Platelets revealed 10 bands (pH 7.5-5.8), and erythrocytes, 5 ill-defined bands (pH 5.6-4.9). These data illustrate the diversity of the lysosomal acid esterase isoenzymes of the different types of blood cells. The characteristic isoenzyme pattern of acid esterase in T lymphocytes and monocytes is well in line with the cytochemical staining pattern and indicates the existence of cell-specific enzyme variants.

NONSPECIFIC ESTERASE has been extensively studied in normal and neoplastic blood cells by cytochemical and biochemical methods (for review refer to Li et al.1 and Higgy et al.2). Among the multitude of different methods, the so-called acid esterase (AcE) (EC 3.1.1.6) staining using α-naphthyl acetate as substrate and a reaction pH of 5.8 is widely used for combined visualization of monocytes and T lymphocytes.3, 4 In monocytes, the reaction product shows a diffuse distribution,7,8 and in T lymphocytes, a distinct granular pattern localized in a paranuclear area of the cytoplasm.2,3 The typical enzyme activity of monocytes has been widely used for the diagnosis of monocytic leukemia.9 Similarly, the dot-like paranuclear AcE reaction pattern has been increasingly used in the classification of some types of leukemia and malignant lymphoma.5,10,11

The intent of this investigation was to detect the activity and isoenzymes of AcE using isoelectric focusing (IEF). The results of the enzyme activity assays were compared with the cytochemical features of various types of cells. The polymorphism of AcE was studied in order to clarify whether the cytochemical patterns of different types of blood cells are due only to different distributions of identical isoenzymes within the cells, or to a cell-specific pattern of enzyme polymorphism.

MATERIALS AND METHODS

Separation of Cells

Heparinized fresh venous blood gained from 40 healthy donors was utilized throughout the investigation. Thymocytes and B lymphocytes were obtained from cell suspensions of thymus glands and tonsils obtained during heart surgery and tonsillectomy, respectively.

Cell separation was performed according to Boyum12 using a Ficoll-Urografin (Pharmacia, Uppsala, Sweden; Schering, Berlin, West Germany) density gradient (Fig. 1). The contaminating red cells in the bottom fraction were removed by osmotic lysis. Monocytes were separated from nonadherent cells as described by Bennett and Cohn.13 For the purification of T lymphocytes, lymphocyte suspensions were passed through an anti-human immunoglobulin column using Degalan V 26 (polymethylmethacrylic beads, Degussa, Hanau, West Germany) treated with human IgG anti-human rabbit IgG,14 and human C3. Platelets were obtained from platelet-rich plasma by centrifugation (400 g, 20 min, 20°C). Erythrocytes were separated by ultracentrifugation as described by Fenton and Richardson.15

During the isolation of B lymphocytes from minced and sieved tissue specimens of tonsils, T lymphocytes were excluded by incubation of the suspension with 2-aminoethylithiouronium bromide (AET; Sigma, Munich, West Germany) treated sheep erythrocytes,16 followed by repeated centrifugation using a Ficoll-Urografin gradient.17 The sediment containing rosetted cells was discarded. When necessary, monocytes, macrophages, and other tissue-resident cells were removed by glass adhesion (Fig. 2). Purified cell suspensions were used only when the viability rate exceeded 95%, as determined by the dye exclusion method.

The purity of isolated cell suspensions was measured by differential cell counting of cytospin preparations (Shandon, London, England). The naphthol-AS-D-chloroacetate esterase reaction18 was used for selective staining of neutrophils and the AcE reaction for visualization of monocytes and T lymphocytes. T lymphocytes were counted in Thoma New Chambers after rosetting with neuraminidase (Behring, Marburg, West Germany) treated sheep erythrocytes.19,20 The purity of B-lymphocyte populations was checked by direct and indirect immunofluorescence according to the principles.

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Supported in part by the Deutsche Forschungsgemeinschaft SFB 111, Project CL3.

Submitted September 19, 1979; accepted January 28, 1980.

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0006-4971/80/5506-0003 $01.00/0
Enzyme Assay

A quantity of 0.5–1 × 10⁹ cells were suspended in 0.02 M HEPES buffer (Serva, Heidelberg, West Germany) and diluted (1:1 v/v) with 0.5 M sucrose solution. For isolation of lysosomes, cell suspensions were subjected to a cavitation bomb (Parr Instruments) and centrifugation (450 g, 15 min, 4°C). The supernatant was ultracentrifuged at 20,000 g for 20 min at 4°C. The 20,000-g sediment was resuspended in a 1% Triton X-100 solution (Serva) for 30 min and ultracentrifuged at 100,000 g for 60 min at 4°C (Fig. 3). For enzyme assay, α-naphthyl acetate (Sigma) was used as substrate and fast blue RR (Sigma) as coupler. The spectrophotometric measurement was carried out at 500 nm (Radzun et al., in preparation). The enzyme activity was expressed in ml/10⁹ cells or related to the protein content of the specimens estimated by the method of Lowry using a mixture of human serum albumin and fibrinogen (1:1 w/w) as reference protein.

Isoelectric Focusing of Acid Esterase

Isoelectric focusing was performed on 4.3% polyacrylamide thin-layer slabs containing 13.3% (w/v) sucrose, 1% (w/v) Triton X-100, and 0.057% (w/v) riboflavin (Canalco, Rockville, Md.). The pH gradient extended from 3.5 to 10 and was maintained by addition of...
5% (v/v) amphotoline pH 3.5-10 (LKB, Bromma, Sweden), 0.36% (v/v) amphotoline pH 4-6, and 0.36% (v/v) amphotoline pH 5-7. The initial voltage of 200 V was raised to a terminal voltage of 1200 V within 4 hr at 4°C. For each run, a minimum activity of 60 mU was used. The enzyme bands were visualized by the histochemical method of Müller et al. at pH 5.8. α-Naphthyl acetate was used as substrate and hexazotized pararosanilin as coupler. The staining time beyond which no additional effects were detectable was 60 min at 20°C. The gels were evaluated densitometrically at 537 nm (Flying spot TLD-lOO, Vitatron, Cologne, West Germany). The value for the isoelectric point (pI) of each band was determined by measuring the pH of aliquots of gel strips.

RESULTS

Cell Suspensions

Utilizing 600 ml venous blood, the final cell yield for granulocytes ranged from 0.5 to 1.0 × 10⁸, for monocytes from 0.5 to 1.0 × 10⁹, and for T lymphocytes from 1.0 to 3.5 × 10⁸. A total of 0.9 × 10⁹ B lymphocytes could be isolated from 5 tonsils.

Table 1 shows the differential count for each cell suspension. Erythrocytes, platelets, granulocytes, B lymphocytes, thymocytes, and T lymphocytes showed a purity of more than 98%. Monocyte suspensions contained 20% lymphocytes. Since the enzyme activity of monocytes was more than 7 times that of lymphocytes, the relatively high contamination of the monocyte suspensions by lymphocytes can be neglected.

Cellular Enzyme Activity

The values for the activity of AcE in the different cell populations were found to be well in line with the results of cytochemical analyses. Monocytes showed the highest values, with a mean of 39 mU/10⁷ cells. The corresponding values for B and T lymphocytes were 5.5 and 5.3 mU/10⁷ cells, respectively. The cell-count-related enzyme activity in thymocytes had a mean of 8.1 mU. Granulocytes revealed a lower level of enzyme activity, with a mean value of about 1.0 mU/10⁷ cells. The lowest content of AcE was detected in platelets. When the enzyme values were related to the protein concentration of the samples, a different sequence resulted (Table 2).

Isoenzyme Pattern of Acid Esterase

The isoenzyme pattern of AcE for each cell population is summarized in Fig. 4. The pI values of the various enzyme bands referred to in this diagram usually represent mean values of 4-6 different analyses. Individual samples of each type of cell were highly similar, both in the number of bands and in pI value, which exhibited only minor variations never exceeding pH 0.2.

Monocytes showed 4 bands between pH 6.2 and 5.86. The most cathodic band had a pI of 6.2. The next, intermediate pair showed pI values of 6.1 and 6.0. The fourth anodic band was localized at pH 5.86 and accounted for 85% of the total enzyme activity.

The enzyme activity of granulocytes was found to be low. Thus, concentration and pooling of several samples was necessary. The pooled lysosomal fractions of these cells showed 11 bands distributed throughout the pH range of 7.9-4.8. In the cathodic region of the gels, 4 bands were localized between pH 7.9 and 7.5. A solitary band was localized at pH 7.0 and a further group of 5-6 bands, between pH 5.75 and 4.8.

The IEF pattern of T lymphocytes comprised 3 complexes. The cathodic complex showed 1 strong and 3 weak bands between pH 7.9 and 7.6. The intermediate complex contained 5 strong bands between pH 6.4 and 5.9. This complex accounted for a major proportion of the enzyme activity. The anodic complex was made up of 4 bands evenly distributed between pH

<table>
<thead>
<tr>
<th>Separated Cell Population</th>
<th>AcE Activity*</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>mU/1 × 10⁷ Cells</td>
</tr>
<tr>
<td>Monocytes</td>
<td>39.27 ± 3.1 (7)</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>0.96 ± 0.5 (5)</td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>5.33 ± 1.86 (4)</td>
</tr>
<tr>
<td>Thymocytes</td>
<td>5.1 (1)</td>
</tr>
<tr>
<td>B lymphocytes</td>
<td>5.5 ± 3.33 (6)</td>
</tr>
<tr>
<td>Platelets</td>
<td>0.48 ± 0.04 (2)</td>
</tr>
</tbody>
</table>

*Mean ± SD.
†Numbers in parentheses indicate the number of samples.

Table 1. Control of Purity of the Separated Cells Using Cytochemical and Immunologic Tests

<table>
<thead>
<tr>
<th>Separated Cell Population</th>
<th>Differential Cell Count*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monocytes†</td>
</tr>
<tr>
<td>Monocytes</td>
<td>75.6 ± 9.9</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>0</td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>0</td>
</tr>
<tr>
<td>B lymphocytes</td>
<td>0.7 ± 0.9</td>
</tr>
<tr>
<td>Thymocytes</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Expressed in percent (mean ± SD).
†Identified by AcE staining.
‡Identified by morphology and by chloroacetate staining.
§Identified by rosette assay using neuraminidase-treated sheep erythrocytes.
†Identified by staining for surface immunoglobulin and HLA-B (Ia-like antigens).
5.0 and 4.5. Between the intermediate and the anodic complexes, 3 bands of low intensity were detectable.

Thymocytes showed a pattern highly similar to that of T lymphocytes, but lacked two bands, one in the cathodic and one in the anodic complex. The three weak bands found for T lymphocytes between the anodic and intermediate complexes were also missing.

B lymphocytes displayed 7 bands, with a group of 5 localized between pH 6.4 and 5.9 and 2 weak bands at pH 5.6 and 5.5.

Platelets showed 10 bands between pH 7.45 and 5.8, and erythrocytes had 5 ill-defined bands between pH 5.55 and 4.95.

Figure 5 illustrates three typical IEF patterns of different cell populations.

**DISCUSSION**

In cytochemical tests, α-naphthyl acetate esterase has been widely used to selectively visualize blood monocytes and monocyte-derived macrophages.\(^7\)\(^-\)\(^9\)\(^,\)\(^25\) In a majority of the investigations, the reaction was performed at a neutral pH. In later studies, it was mentioned that lymphocytes and erythropoietic cells were also positive for this enzyme. In this context, it became evident that at least the specific cytochemical enzyme patterns encountered in certain types of cells are clearly dependent on pH.\(^5\)\(^,\)\(^26\) At a low pH, it was found not only that monocytes showed diffuse strong activity, but also that a majority of blood lymphocytes contained a dot-like paranuclear reaction product. These lymphocytes have been shown to represent the main proportion of the T-lymphocytes subpopulation.\(^3\)\(^,\)\(^5\)\(^,\)\(^6\)

In the present investigation, various types of blood cells (including T lymphocytes), tonsil B lymphocytes, and thymic lymphocytes were isolated and subjected to biochemical enzyme assay. The results were found to be mainly in line with the cytochemical findings. Monocytes showed the highest values, exceeding those of lymphocytes by a factor of 7. There were no significant differences among the values of T lymphocytes, B lymphocytes, and thymocytes. In this respect, the results appeared to differ from the cytochemical findings. With cytochemical staining, blood and tonsil B lymphocytes exhibit a negative reaction or merely small granules of weak activity, whereas the vast majority of blood and tonsil T lymphocytes are characterized by a strong dot-like paranuclear enzyme reaction product.\(^1\)\(^1\) On electron microscopic examination, lysosomes of B lymphocytes are evenly distributed throughout the cytoplasm.\(^27\) In T lymphocytes, on the other hand, there is a paranuclear accumulation of lysosomes aggregated in the immediate vicinity of lipid-containing vacuoles. The dot-like paranuclear reaction product found in a majority of blood T lymphocytes is seen in a minor proportion of thymocytes—Kulenkkampff et al.\(^5\) found this pattern in 3% and Ranki et al.\(^6\) in 13% of thymic lymphocytes. The remaining proportion of thymocytes shows no AcE activity, or a diffuse distribution of very weak activity,
ACID ESTERASE ISOENZYMES IN BLOOD CELLS

Fig. 5. Three representative examples of the IEF pattern of lysosomal AcE from separated cells: blood monocytes (A), tonsil B lymphocytes (B), and blood platelets (C).

depending on the staining conditions. In agreement with the cytochemical findings, granulocytes exhibit an extremely low level of enzyme activity.

The clear differences in the level of enzyme activity, as well as the characteristic cytochemical patterns of different types of cells, raised the question as to whether these differences might be due to different isoenzymes specific to certain cell types. If such isoenzymes exist, it would be possible to gain new cytogenetic information from the study of normal and neoplastic conditions. The phenotypic analysis of enzyme polymorphism might provide a means of classifying neoplastic cell lines that have hitherto unclassifiable. Specific enzyme polymorphism has often been used to ascertain the derivation of certain cells from progenitors that are not otherwise identifiable as such.

The polymorphism of AcE in different types of blood cells revealed clear differences among the various cell types. The most striking results were obtained in monocytes. These cells, unlike all other types of blood cells, showed four distinct bands between pH 6.2 and 5.86. The most anodic band accounted for more than 85% of the total enzyme activity, which suggests that the high cytochemical enzyme activity seen in blood monocytes is at least 85% due to this specific isoenzyme with a distinct pI of 5.86. No other blood cell population revealed such an isoenzyme pattern. Granulocytes had 11 bands distributed throughout the gel. T lymphocytes could be easily distinguished from B lymphocytes, as well as from all other cell lines, by the typical distribution of their isoenzymes in three distinct complexes. Thymocytes were most similar to T lymphocytes in IEF pattern. This finding is particularly remarkable, because T lymphocytes and thymocytes clearly differ in enzyme cytochemical staining pattern: with the exception of a few cells, thymocytes demonstrate no, or merely diffuse, weak activity of AcE, whereas a majority of blood T lymphocytes contain the typical dot-like reaction product. In T lymphocytes, two to three bands of extremely low intensity were occasionally seen around pH 5.4. This irregular incidence could not be related to contamination by other types of cells. It appears to be very probable that the irregular bands were induced by the presence of subpopulations of T lymphocytes occurring with a variable frequency in the blood. Under normal conditions, the number of cells belonging to these subpopulations is low, and thus their isoenzymes remain below the limit of resolution.

To our knowledge, the literature does not contain any reports on direct AcE assays of blood cells. There are also only a few publications concerning the polymorphism of AcE determined by polyacrylamide disc
electrophoresis. Li et al. studied the polymorphism of AcE in human blood leukocytes: monocytes showed three bands and lymphocytes two bands. Young et al. applied IEF for the detection of AcE isoenzymes in homogenates from various normal organs, tumor tissue, and erythrocytes. They described 18 bands between pH 8.3 and 4.2. Except for erythrocytes, however, it was not possible to relate certain bands to a specific cell type in their study. Thus, the present article is the first report on the polymorphism and IEF pattern of AcE isoenzymes investigated in purified blood cell populations. Our study disclosed that a cell-specific IEF pattern of AcE can be demonstrated for all the types of blood cells we analyzed.

Although AcE has mainly been used in cytochemical tests to differentiate monocytes or T lymphocytes, the results of our study show that this enzyme is easily detectable in practically all blood cells. The occurrence of high enzyme activity in some cases of plasma cytoma and hairy cell leukemia has already been well documented. Sundström et al. gave an account of AcE activity in cultured lines of malignant B-cell lymphomas.

The available data clearly reveal that the various types of blood cells differ in concentration, distribution, and isoenzyme composition of AcE. The results of our investigation further suggest that there are at least some isoenzymes that might be specific to certain types of cells. It is highly probable that one candidate is the anodal isoenzyme of monocytes with a pl of 5.86. To test this possibility, we have started to prepare a monospecific antiserum to AcE isoenzyme 5.86. If the isoenzyme 5.86 proves to be antigenically specific to monocytes, then the anti-AcE isoenzyme 5.86 serum would be a monocyte-specific antiserum. Such an antiserum would be highly useful, since, to our knowledge, there is no antiserum that is specific for cytoplasmic constituents of monocytes. For example, an antiserum specific for cytoplasmic constituents of monocytes would provide a way to selectively immunostain reactive and neoplastic monocytes, and probably their derivatives, in hitherto unclassifiable tumors.

**REFERENCES**

Lysosomal acid esterase: activity and isoenzymes in separated normal human blood cells

HJ Radzun, MR Parwaresch, C Kulenkampff, M Staudinger and H Stein