Expression of 5′-Nucleotidase (CD73) Related to Other Differentiation Antigens in Leukemias of B-Cell Lineage


Ecto-5′nucleotidase (5′NT; CD73) expression was studied with a monoclonal antibody (7G2) and a radiochemical assay and compared with the expression of other antigens in B-cell-lineage leukemias on cells from 100 leukemic patients and two cell lines. A B-cell origin was confirmed by the expression of CD19 and HLA-DR. Four stages of B-cell leukemias were defined: stage I (pro-B) as CD10+, cytoplasmic μ- (cp-), surface Ig- (slg-); stage II (CALL) as CD10+/cp-/slg-; stage III (pre-B) as CD10+/cp+/slg-; and stage IV (B) as CD10+/cp+/slg+. A linear correlation was found between immunohistochemical and radiochemical determination of 5′NT (r = .86). 5′NT expression was low in T-cell leukemias and stage I, high in stages II and III, and low again in stage IV of B-cell leukemias. 5′NT expression was not related to cp-.

MATERIALS AND METHODS

Reagents. The CD3, CD4, CD7, CD8, CD10, and CD34 MoAbs were purchased from Sanbio (Uden, The Netherlands); CD2, HLA-DR, κ, and λ from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (Amsterdam, The Netherlands); CD19, CD22, and terminal deoxynucleotidyl transferase (TdT) from DAKO (ITK Diagnostics, de Kwakel, The Netherlands); CD20 from Becton Dickinson (Etten-Leur, The Netherlands); CD21, CD22, CD34, and terminal deoxynucleotidyl transferase (TdT) from DAKO (ITK Diagnostics, de Kwakel, The Netherlands); CD20 from Becton Dickinson (Etten-Leur, The Netherlands); and CD21 from Coulter Clone (Hialeah, FL). The CD73 antibody used was 7G2.12 Fluorescein isothiocyanate (FITC)-conjugated antimouse IgG was from DAKO. [8-3H]-inosine monophosphate (IMP; 50 μCi/mL, specific activity 80 mCi/mmol) was derived from Amersham International (Buckinghamshire, UK); α,β-methylene adenosine diphosphate (AOPCP), IMP, inosine, hypoxanthine, and adenosine from Sigma (St Louis, MO). Plastic sheets precoated with 0.1 mm polyethyleneimine (PEI)-cellulose were from Merck (Darmstadt, Germany).

Cells. Cells were obtained from bone marrow and peripheral blood samples of 100 leukemic patients, 97 patients with ALL, and three with chronic lymphocytic leukemia (CLL). The study was approved by the Institutional Review Board. Informed consent was obtained in each case according to the Declaration of Helsinki. In addition, two lymphoblastic cell lines, WI-L2 and 1254, were used.11 Mononuclear cells were isolated on a Ficoll Isopaque gradient and in most cases used after cryopreservation as described before.11

Immunohistochemistry. Immunoperoxidase staining was performed using cryopreserved cells to detect cp-. Aliquots of 50 μL of 0.5 × 109 cells/mL. Earle's balanced salt solution with 5% human serum albumin were cytocentrifuged and dried on silicon-gel. Cells were

From www.bloodjournal.org by guest on October 3, 2017. For personal use only.
fixed in buffered formaldehyde acetone (4°C) for 30 seconds. After rinsing with water and phosphate-buffered saline (PBS), cells were incubated with 50 μL of MoAbs at room temperature for 60 minutes. Next, the slides were rinsed with PBS three times. Rabbit antimonouse peroxidase with 2% pooled human serum was added for 60 minutes. Peroxidase staining was performed using diamino benzidine with 0.05 mol/L imidazole in 0.05 mol/L Tris buffer (pH 7.6) for 10 minutes, and with copper sulphate 0.5% for 5 minutes. Cells were counterstained with hematoxylin.

For detection of surface antigens, cells were fixed with 1% paraformaldehyde diluted in PBS for 5 minutes followed by rinsing with PBS containing 0.1% bovine serum albumin (BSA) and 0.02% sodium azide. Immunofluorescence staining was performed with MoAbs CD2, CD3, CD4, CD7, CD8 (as T-cell markers), CD10, CD19, CD20, CD21, CD22, CD34, TdT, HLA-DR, γ, δ, and CD73. After rinsing twice, FITC-conjugated rabbit antimonouse IgG with 2% pooled human serum and 10% rabbit serum was added for 30 minutes. Cells were rinsed twice and resuspended in 0.3 mL PBS/BSA/sodium azide. Fluorescence was measured with a FACStar (Becton Dickinson). Flow-cytometric detection of intracellular TdT was performed after fixation of the cells with buffered formaldehyde acetone as described by Slaper-Cortenbach et al.13

Definitions. Samples were considered antigen positive if the percent of cells expressing the antigen was greater than 20% after subtracting control. The samples of the B-cell-lineage leukemias were classified into four stages. The B-cell origin of these samples was confirmed by virtue of the expression of CD19 and HLA-DR. The most mature, stage IV B-cell leukemias were CD10+, cp+, and sIg-. Less mature, early pre-B cells that did not express cp were subdivided in two stages based on the expression of CD10 according to the model of Nadler et al.6: stage II (CALL) cells CD10+/cp+/sIg-; stage I (pro-B-ALL) cells CD10+/cp-/sIg-. A good linear correlation was found between the percentage of 5'NT+ cells determined by the FACS and the 5'NT activity determined by the radiochemical assay (r = .86; r = .84 if only B cells were evaluated; Fig 1). The percentage of 5'NT+ cells is related to the immunologic subtypes, as shown in Table 1 and Fig 2. The radiochemical 5'NT activity of the different immunologic subtypes is shown in Table 1 and Fig 3. It is clear that in the least mature stage I the 5'NT expression is low. It is high in the CD10− stages II and III and low again in stage IV. 5'NT was low in T-ALL, including one CD10− T-ALL.

We evaluated whether there was correlation between the expression of 5'NT and other differentiation antigens of the B-cell lineage. No significant correlation could be detected between the percent of cells positive for 5'NT and the percent of cells positive for CD20, CD21, CD22, CD34, or TdT. For CD19 and HLA-DR, such a correlation was not performed because all B-lineage samples were positive for these two antigens.

There was a significant correlation between the percent of CD10− and 5'NT− cells (P < .05; Table 2). The mean (±SD) 5'NT activity of CD10− samples was 6.1 ± 12.2

---

**Table 1. Percentages of 5'NT+ Cells Determined by Immunofluorescence Staining and 5'NT Activity Determined Radiochemically in T-ALL and the Four Stages of B-Cell-Lineage Leukemias**

<table>
<thead>
<tr>
<th>Immunophenotype</th>
<th>5'NT%</th>
<th>5'NT (nmol/h/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T-ALL</strong></td>
<td>2.0</td>
<td>0.0-9.1 0.8 0.0-2.6</td>
</tr>
<tr>
<td><strong>B-lineage:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I. pro-B</td>
<td>1.5</td>
<td>0.6-9.4 3.0 1.9-6.0</td>
</tr>
<tr>
<td>Stage II. CALL</td>
<td>38.3</td>
<td>0.4-91.0 30.0 0.0-109.9</td>
</tr>
<tr>
<td>Stage III. pre-B</td>
<td>48.4</td>
<td>6.9-93.2 23.2 1.6-82.5</td>
</tr>
<tr>
<td>Stage IV. B</td>
<td>5.3</td>
<td>0.8-31.5 0.6 0.0-11.7</td>
</tr>
</tbody>
</table>

---

RESULTS

There were 19 T-ALL samples and 83 samples of B-cell lineage including the two cell lines. Four samples were...
nmol/h/10⁶ cells (median 1.8) compared with 36.9 ± 28.8 nmol/h/10⁶ cells (median 41.4) of CD10⁺ samples. The range of 5'NT values in the CD10⁺ stages II and III is very wide, as is illustrated in Table 1 and Figs 2 and 3; several CD10⁺ cases were even 5'NT⁻. High 5'NT activities were not found in stages I and IV, but were always associated with CD10 expression. Two-color fluorescence studies have been performed in a few cases (Fig 4). These confirm the relationship between 5'NT and CD10 expression. Cases that were CD10⁺ cp⁻ and cases that were CD10⁺ cp+ did not show significant differences in 5'NT expression. Among stage III samples, the number of CD10⁺ cp⁻ samples was too low (n = 3) to compare their 5'NT activity with that of CD10⁺ cp⁺ cases.

Within the CD10⁺ group, there was no significant correlation between the percent of cells positive for 5'NT and the percent of cells positive for cp, CD20, CD21, CD22, CD34, and TdT. A significant inverse correlation between the percent of cells positive for κ or λ and the percent of cells positive for 5'NT was found (P < .01; Table 2). The mean (±SD) 5'NT activity of κ⁺ samples was 35.7 ± 28.8 nmol/h/10⁶ cells (median 36.0) compared with 2.2 ± 3.9 nmol/h/10⁶ cells (median 0.6) of κ⁻ samples. The three CLL samples were κ⁺ or λ⁺ and were therefore classified as stage IV B-cell leukemias. The percentages of 5'NT⁺ cells of these samples were 31.5%, 0.8%, and 1.4%. In the latter two cases, 5'NT activity was determined radiochemically: 0.9 and 0.1 nmol/h/10⁶ cells. These data are also shown in Figs 2 and 3.

Cell line 1254 is a spontaneous mutant of WI-L2. 1254 shows a more mature phenotype with κ expression and is 5'NT⁻, opposite to WI-L2 cells, which are κ⁻ and 5'NT⁺.

**DISCUSSION**

Up to now the relationship of 5'NT to B-cell maturation, in particular in the very early stages of B-cell development, has not been clearly defined. In this study we established the place of 5'NT in the maturation scheme by comparing its expression, determined by immunocytochemical and radiochemical assays, in relation to a panel of other differentiation antigens. Leukemic B-cell-lineage samples were categorized into four stages depending on their expression of κ or λ and CD10. Stage I (pro-B) samples did not express any of these three markers. The B-cell origin of these samples was confirmed by the expression of CD19 and HLA-DR. According to the model of Nadler et al., pro-B cells are of a less mature type than the cells that already express CD10 and that were called stage II in the

**Table 2. Relation Between 5'NT Expression and CD10 and κ-λ Expression in B-Cell-Lineage Leukemias**

<table>
<thead>
<tr>
<th>5'NT⁺ cells</th>
<th>&lt;20%</th>
<th>&gt;20%</th>
<th>Total</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD10⁺ cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20%</td>
<td>8</td>
<td>5</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>&gt;20%</td>
<td>9</td>
<td>28</td>
<td>37</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>33</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>κ⁺ cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20%</td>
<td>11</td>
<td>32</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>&gt;20%</td>
<td>7</td>
<td>1</td>
<td>8</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>33</td>
<td>51</td>
<td></td>
</tr>
</tbody>
</table>

The number of antigen-positive (>20% of the cells expressing the antigen) and antigen-negative (<20% of the cells expressing the antigen) cases is given.
present study. Normal counterparts for leukemic pro-B cells have been recognized among fetal and adult bone marrow cells. Our results clearly show that the median 5′NT is low or absent in the stage I pro-B cells, increases to very high activities in the CD10+ stages II and III, and decreases again in stage IV. 5′NT is only expressed on the cell surface together with CD10, but is clearly not present on all CD10+ leukemias. This finding is reflected in the wide ranges of 5′NT activities in stages II and III. In conclusion, 5′NT is expressed in CD10+ stages and its activity decreases before or at λ appears on the cell surface.

Our findings are based on a study of malignant cells and it can be questioned whether the results can be extrapolated to normal B cells. A study to determine the 5′NT activity of non-malignant CD10+ cells has to show whether the high 5′NT activity on leukemic CD10+ cells is a malignant phenomenon or a normal maturation phenomenon. In a previous report we have used a histochemical lead staining method that is less sensitive than the methods used in the present report to study the prognostic significance of 5′NT in a group of 33 children with CD10+ ALL. It appeared that a high 5′NT activity was related to a poorer prognosis among children with CD10+ ALL. This prognostic factor was found to be independent from sex, age, and white blood cell count. Very recently, other investigators confirmed the prognostic significance of 5′NT activity among adults with CD10+ ALL.

Several hypotheses have been postulated to explain the prognostic value of 5′NT by its possible role in resistance to the thiopurines 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG) or to methotrexate. If 5′NT plays an important role in drug resistance, one could expect an increased sensitivity of T-cell leukemias to methotrexate or thiopurines. The sensitivity to these drugs depends, however, on many other factors that might differ between T-ALL and B-lineage ALL. For example, the activity of hypoxanthine-guanine phosphoribosyl transferase (HG-PRT), the enzyme that converts the inactive 6-MP and 6-TG to their cytotoxic nucleotides, is lower in T-ALL than in B-lineage ALL.

Recent findings suggest that 5′NT plays a role in signal transduction pathways and lymphocyte proliferation. CD73 antibodies stimulated DNA synthesis specifically in 5′NT+ lymphocytes. Also, CD73 antibodies induced a fraction of normal B cells to leave G0 phase and enter the G1 phase of the cell cycle. The same antibodies stimulated H-thymidine uptake in malignant B cells from patients with CLL. Thus, 5′NT+ CD10+ ALL cells might have different control mechanisms of cell proliferation than 5′NT- CD10+ ALL cells. The present study did not show differences in expression of other B-cell antigens between 5′NT+ CD10+ and 5′NT- CD10+ ALL.

We conclude that the place of 5′NT (CD73) in leukemias corresponding to stages of early B-cell development has been characterized. Future studies should make clear whether a high expression of this enzyme in CD10+ leukemias is a normal maturation phenomenon or a malignant phenomenon. The role of 5′NT in drug resistance and cell proliferation has to be studied.

REFERENCES

12. Thompson LF, Ruedi JM, Moldenhauer G, Moller P, Low MG, Klemens MR, Massaia M, Lucas AH: Production and characterization of monoclonal antibodies to the glycosyl phosphati-
diyinositol-anchored lymphocyte differentiation antigen ecto-5′-nucleotidase (CD73). Tissue Antigens 35:9, 1990


Expression of 5’-nucleotidase (CD73) related to other differentiation antigens in leukemias of B-cell lineage

R Pieters, LF Thompson, GJ Broekema, DR Huismans, GJ Peters, ST Pals, E Horst, K Hahlen and AJ Veerman