Unique Pattern of Nuclear TdT Immunofluorescence Distinguishes Normal Precursor B Cells (Hematogones) From Lymphoblasts of Precursor B-Lymphoblastic Leukemia

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Abstract

Normal precursor B cells or hematogones share morphologic and immunophenotypic similarities with lymphoblasts of precursor B-lymphoblastic leukemia. The numbers are often increased and difficult to distinguish in many patients following chemotherapy for precursor B-lymphoblastic leukemia. The purpose of this study was to establish a unique method for differentiating hematogones from lymphoblasts by evaluating the immunofluorescence pattern of nuclear terminal deoxynucleotidyl transferase (TdT) staining in 29 cases of TdT+ acute leukemia and 20 cases with increased hematogones. All 29 cases of TdT+ acute leukemia demonstrated a finely granular pattern of TdT immunofluorescence that was uniformly distributed in the nucleus, whereas all 20 cases with increased hematogones demonstrated a coarsely granular or speckled pattern of TdT immunofluorescence, which often intensely aligns the nuclear membrane. The nuclear pattern of immunofluorescence using antibodies to TdT is an effective method for distinguishing hematogones from leukemic blasts.

Materials and Methods

Case Selection

We evaluated specimens from 40 patients seen in the Special Hematology Laboratory, Hartford Hospital, Hartford, CT. The specimens included 40 bone marrow aspirate samples and 5 peripheral blood samples from 29 cases of TdT+ acute leukemia and 20 cases with increased hematogones

Table I.

Normal precursor B cells, or hematogones, are commonly observed in relatively low percentages of bone marrow specimens in children and, occasionally, in adults.1,2 Increased numbers of hematogones may also be present in children and adults in a variety of clinical conditions including Hodgkin and non-Hodgkin lymphoma, metastatic pediatric tumors, and acquired and congenital immune cytopenias.1-6 Increased numbers of hematogones are also frequently observed in recovery bone marrow samples following chemotherapy and bone marrow transplantation.3,4,7 Normal precursor B cells often share morphologic and immunophenotypic similarities with lymphoblasts of precursor B-lymphoblastic leukemia.1-4 The 2 cell types may be difficult to distinguish. In patients with precursor B-lymphoblastic leukemia, distinguishing normal precursor B cells from a residual leukemic blast population following chemotherapy may also be problematic, frequently requiring multiparameter flow cytometric analysis. We describe the application of a simple indirect immunofluorescence test for terminal deoxynucleotidyl transferase (TdT) that can be reliably used to distinguish normal precursor B cells, or hematogones, from leukemic blasts.
The diagnosis in all 29 cases of acute leukemia and all 20 cases with increased numbers of hematogones was based on combined morphologic and multiparameter flow cytometric immunophenotypic findings. The 40 patients included 21 males and 19 females. The patients’ ages ranged from 1 to 78 years. For male patients, the age range was 1 to 78 years and for female patients was 2 to 73 years. The mean age for all patients was 21 years.

Morphologic Review

The peripheral blood and bone marrow aspirate smears were stained with Wright-Giemsa and reviewed by 4 hematopathologists (M.T.H., J.A.D., B.J.S., and W.N.R.). Morphologically, the hematogones ranged in size from small to large lymphoid cells with highly condensed, uniform chromatin; absent to inconspicuous nucleoli; and scant, agranular basophilic cytoplasm. The leukemic blasts were mostly medium sized with finely dispersed chromatin; inconspicuous to prominent, often multiple nucleoli; and scant basophilic cytoplasm. Auer rods and azurophilic granules were present in some cases of acute myeloid leukemia.

Flow Cytometric Immunophenotyping, Cell Isolation, and Staining Procedures

Peripheral blood and bone marrow suspensions were diluted, as needed, to a concentration of \(5 \times 10^3/\text{mm}^3\) with phosphate-buffered saline (PBS)-azide and 5% albumin (Sigma Chemicals, St Louis, MO). Cell suspensions containing \(5 \times 10^3/\text{mm}^3\) cells were incubated for 15 minutes with the appropriate fluorochrome-labeled monoclonal antibodies. Fluorochromes for 3-color flow cytometry included fluorescein isothiocyanate (FITC), phycoerythrin (PE), and peridinin chlorophyll protein (PerCP). Fluorochromes for 4-color flow cytometry included FITC, PE, PerCP, and allophycocyanin. RBCs were lysed using the Immunoprep reagent system (Beckman Coulter, Miami, FL) for 3-color flow cytometry and FACSlision (Becton Dickinson Immunocytometry Systems, San Jose, CA) for 4-color flow cytometry. We acquired 5- and 6-parameter (3- and 4-color) data using a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems). The flow cytometer was calibrated daily using Calibrite beads with FACScComp software (Becton Dickinson Immunocytometry Systems). All analysis was performed using Paint-a-Gate software (Becton Dickinson Immunocytometry Systems). The fluorochrome-conjugated monoclonal antibodies used in the study are listed in Table 2.

Immunofluorescence Assay for TdT

Indirect immunofluorescence assays for TdT were performed on Cytospin (Thermo Scientific, Waltham, MA) smears prepared from cell suspensions of bone marrow aspirates, peripheral blood, and cerebrospinal fluid. Ammonium chloride was used to remove RBCs as necessary. Positive control samples (Supertech, Bethesda, MD) were run in combination with the patient’s Cytospin smears. Positive and negative Cytospin smears were processed on the patient and positive control smears. The patients’ Cytospin smears and positive Cytospin smears were incubated at room temperature in a humidity chamber for 30 minutes with a 1:6 dilution of the primary rabbit anti-TdT antibody (Supertech). Following incubation, the Cytospin smears were washed twice with 2 changes of PBS during a 10-minute period to remove excess antibody. The negative Cytospin smears were incubated in the same manner with

Table 2

<table>
<thead>
<tr>
<th>Composition of TdT+ Cases*</th>
<th>No. of Cases</th>
</tr>
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<tbody>
<tr>
<td>TdT+ acute leukemia</td>
<td>29</td>
</tr>
<tr>
<td>Precursor B-lymphoblastic leukemia</td>
<td>20</td>
</tr>
<tr>
<td>Precursor T-lymphoblastic leukemia</td>
<td>1</td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td>6</td>
</tr>
<tr>
<td>Chronic myeloid leukemia in precursor B-lymphoblastic blast crisis</td>
<td>2</td>
</tr>
<tr>
<td>Cases with increased hematogones</td>
<td>20</td>
</tr>
<tr>
<td>History of precursor B-lymphoblastic leukemia</td>
<td>7</td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td>2</td>
</tr>
<tr>
<td>No hematologic malignancy</td>
<td>10</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>1</td>
</tr>
<tr>
<td>Fanconi anemia</td>
<td>2 (brothers)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>1</td>
</tr>
<tr>
<td>Sideroblastic anemia</td>
<td>1</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>3</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>1</td>
</tr>
<tr>
<td>Anemia</td>
<td>1</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>1</td>
</tr>
</tbody>
</table>

TdT, terminal deoxynucleotidy transferase.  
* Some patients may be represented in both categories.
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Table 2
Fluorochrome-Conjugated Monoclonal Antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Manufacturer</th>
</tr>
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<tbody>
<tr>
<td>3-color (hematogones) CD10, CD19, CD20</td>
<td>Beckman Coulter, Miami, FL</td>
</tr>
<tr>
<td>4-color (hematogones) CD10, CD19, CD20, CD45</td>
<td>Becton Dickinson Immunocytometry Systems, San Jose, CA</td>
</tr>
</tbody>
</table>

TdT, terminal deoxynucleotidyl transferase.

A 1:20 dilution of normal rabbit serum. The identical procedure was repeated for the secondary antibody, Supertech F(ab′)_2 goat antirabbit antibody. FITC-conjugated slides were counterstained for 1.5 minutes with a solution containing 100 µL of Eriochrome Black (Panbio, Columbia, MD) in 6 mL of PBS and then washed with PBS for 1 minute. Coverslips were applied using buffered PBS-glycerin.^

All slides were examined under fluorescence light using a mercury-100 fluorescence generator coupled to an Olympus BH2 microscope (Olympus, Center Valley, PA).

Results

Flow Cytometry Interpretation

The normal precursor B cells (hematogones) demonstrated very low right-angle light scatter and low forward light scatter. The hematogones demonstrated slightly dimmer expression for CD45 than normal lymphocytes and a variable spectrum of expression for CD10, CD19, CD20, and TdT.

Nuclear TdT Immunofluorescence Pattern

The nuclear pattern of TdT immunoreactivity was evaluated by 4 hematopathologists (M.T.H., J.A.D., B.J.S., and W.N.R.) in 5 high-power fields. A positive reaction was demonstrated by the appearance of nuclear fluorescence when viewed with a fluorescent microscope. A negative result was indicated by a lack of fluorescence under identical conditions. A nonspecific result was indicated by cytoplasmic staining and was most often seen in granulocytes. All 20 cases with increased numbers of hematogones demonstrated...
a characteristic coarsely granular or speckled pattern of TdT immunofluorescence, which often intensely aligned the nuclear membrane Image 3 and Image 4. All 29 cases of acute leukemia demonstrated a finely granular pattern of TdT immunofluorescence that was uniformly distributed in the nucleus Image 5 and Image 6.

**Discussion**

In patients treated for precursor B-lymphoblastic leukemia, distinguishing hematogones from a minimal residual leukemic blast population may be problematic because hematogones and lymphoblasts of precursor B-lymphoblastic leukemia often share morphologic and immunophenotypic similarities. In

**Image 3** Hematogones with coarsely granular or speckled pattern of terminal deoxynucleotidyl transferase (TdT) immunofluorescence, which often intensely aligns the nuclear membrane (green). Immunofluorescence with antibody to TdT. Cell suspension, bone marrow aspirate (×1,000).

**Image 4** Hematogones with coarsely granular or speckled pattern of terminal deoxynucleotidyl transferase (TdT) immunofluorescence, which often intensely aligns the nuclear membrane (green). Immunofluorescence with antibody to TdT. Cell suspension, bone marrow aspirate (×1,000).

**Image 5** Acute myeloid leukemia with a finely granular pattern of terminal deoxynucleotidyl transferase (TdT) immunofluorescence, which is uniformly distributed in the nucleus (green). Immunofluorescence with antibody to TdT. Cell suspension, bone marrow aspirate (×1,000).

**Image 6** Precursor B-lymphoblastic leukemia with a finely granular pattern of terminal deoxynucleotidyl transferase (TdT) immunofluorescence, which is uniformly distributed in the nucleus (green). Immunofluorescence with antibody to TdT. Cell suspension, bone marrow aspirate (×1,000).
the early stages following bone marrow transplantation, there may be a predominance of hematogones. At levels of 5% or more, hematogones may also be confused with lymphoblasts of precursor B-lymphoblastic leukemia. However, this distinction can be readily accomplished in the majority of cases by multiparameter 3- or 4-color flow cytometry. In this multiparameter flow cytometric study, the hematogones expressed a continuous and complete maturation spectrum of normal B-cell development, whereas the lymphoblasts of precursor B-lymphoblastic leukemia typically deviated from the normal maturation pathway. In normal B-cell development, hematogones can be further divided into a small proportion of very immature (CD10+/CD34+/TdT+) and intermediately differentiated (CD10+/CD34+/TdT−) subsets.

This study evaluated and compared the nuclear pattern of TdT immunofluorescence of patients with hematogones or increased normal precursor B cells with TdT+ blasts of precursor B-lymphoblastic leukemia, some cases of acute myeloid leukemia, and a few cases of precursor T-lymphoblastic leukemia. The normal B-cell precursors demonstrated a characteristic coarsely granular or speckled pattern of TdT immunofluorescence, which often intensely aligned the nuclear membrane. In contrast, leukemic blasts in all cases of TdT+ leukemia demonstrated a finely granular pattern of TdT immunofluorescence that was uniformly distributed in the nucleus. To our knowledge, this unique morphologic pattern of nuclear fluorescence in normal B-cell precursors has not been previously reported.

Previous authors have also reported various methods for distinguishing hematogones and lymphoblasts of precursor B-lymphoblastic leukemia by immunophenotypic techniques. Studies by Farahat et al using quantitative flow cytometry have demonstrated quantitative differences in the number of TdT molecules per cell, with normal precursor B cells having a significantly higher level of TdT expression compared with B-lineage blasts in acute lymphoblastic leukemia. By multiparameter flow cytometry, Weir et al showed quantitative differences in light scatter and intensity of antigen expression with clusters of lymphoblasts separated from the normal templates of hematogones. Rimsza et al, using flow cytometry, identified marked heterogeneity of expression of the adhesion molecules (CD44 and CD54) in hematogone-rich cases compared with acute lymphoblastic leukemia cases. Other investigators have also reported various techniques using immunohistochemical techniques for distinguishing hematogones and lymphoblasts of precursor B-lymphoblastic leukemia. In 1998, Rimsza et al demonstrated that 1 cluster of 5 or more CD34+ or TdT+ cells by immunohistochemical analysis could identify patients at risk for relapse. They concluded that this method could reliably be used to differentiate hematogones from neoplastic lymphoblasts. They also reported increased numbers of CD20+ cells in patients with hematogones as compared with the lymphoblasts of precursor B-lymphoblastic leukemia.

Evaluation of the pattern of TdT immunofluorescence provides a very useful tool for distinguishing normal B-cell precursors from leukemic blasts. This test may provide useful supplemental information to standard morphologic and multiparameter flow cytometric data. In select cases, this may be the only test necessary to confirm the presence of a population of normal precursor B cells. For example, in a patient with clinically suspected immune thrombocytopenic purpura with numerous immature lymphoid cells identified morphologically, a TdT study by immunofluorescence may be used to confirm the presence of a population of normal B-cell precursors, or hematogones. This technique may also provide valuable clinical data when flow cytometry cannot be used. For example, TdT immunofluorescence may be used in the microscopic examination of cerebrospinal fluid when only small numbers of lymphoid cells are present in the specimen. This technique may be used to initially diagnose involvement of the cerebrospinal fluid by precursor B-lymphoblastic leukemia and for following up patients for relapse of precursor B-lymphoblastic leukemia.

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References


