Reactivity of Acute Lymphoblastic Leukemia and Normal Bone Marrow Cells with the Monoclonal Anti-B-Lymphocyte Antibody, Anti-Y 29/55

Andreas Hirt, Christoph Baumgartner, Hansjörg K. Forster, Paul Imbach, and Hans P. Wagner

ABSTRACT

Malignant lymphocyte populations in peripheral blood of patients with B-cell chronic lymphocytic leukemia, leukemic variant of B-cell non-Hodgkin's lymphoma, and hairy cell leukemia can be characterized by the use of a monoclonal murine antibody (anti-Y 29/55) which is directed against a cell membrane component normally confined to the sessile nonrecirculating cells of the B-lymphocyte population in lymphoid tissues. The present report describes the reactivity of the anti-Y 29/55 antibody with bone marrow cells obtained from children with acute lymphoblastic leukemia using an indirect immunofluorescence method in combination with morphological and cytokinetic studies. In 25 patients (acute lymphoblastic leukemia subtype: 14 common; 4 pre-B-cell; 4 null; and 3 T-cell), a maximum of 2% of cells (small lymphocytes) were stained. One patient presented with blasts exhibiting cytoplasmic and surface immunoglobulin M (IgM) (pre-B-B-cell acute lymphoblastic leukemia). About 11% of this patient's blasts cells showed a positive reaction with anti-Y 29/55. They could not be differentiated by morphological criteria from the anti-Y 29/55-negative blast cell population. In another patient with pre-B-B-cell acute lymphoblastic leukemia, only 1% of anti-Y 29/55-positive cells was found.

In bone marrow of children with relative lymphocytosis, 1.4 to 8.7% of mononuclear cells reacted with anti-Y 29/55. Morphologically, these cells were small lymphocytes and predominantly expressed surface IgM. In two of these children, a further subdivision of bone marrow cells could be achieved by combining anti-Y 29/55 and cytoplasmic IgM reactivity with [3H]thymidine pulse labeling. These studies revealed that the actively proliferating, normal pre-B-cell population was anti-Y 29/55-negative, whereas a nonproliferating population of anti-Y 29/55-reactive, cytoplasmic IgM-positive cells probably represented B-cells with surface immunoglobulin M reacting when cytoplasmic IgM was assessed. We conclude that the reactivity of the monoclonal anti-B-cell antibody (anti-Y 29/55) is restricted to surface immunoglobulin-positive bone marrow cells and that neither leukemic or normal pre-B-cells nor common, null-cell, or T-cell acute lymphoblastic leukemia blasts react.

INTRODUCTION

Recently, a monoclonal murine antibody (anti-Y 29/55) with specificity for a human B-leukemia-associated antigen has been described (3). This antibody reacts with leukemic B-lymphocytes obtained from adult patients with chronic lymphocytic B-cell leukemia, B-cell non-Hodgkin's lymphoma, and hairy cell leukemia (3, 5). Thus, the monoclonal antibody recognizes malignant cell populations which derive from B-cells arrested at various stages of differentiation. This marker of B-cell differentiation was also found on normal B-lymphocytes in secondary lymphoid organs like lymph nodes, spleen, and tonsils. Only a minority of recirculating B-cells in peripheral blood and of cells from bone marrow preparations of normal adult donors express the antigen.

In bone marrow of children with ALL(2) in remission, a relatively high percentage of pre-B-cells may occur after cessation of long-term chemotherapy (8). Moreover, juvenile ALL patients may have blast cell populations corresponding to B-cell precursors (2, 4, 11). For this reason, the reactivity of the monoclonal antibody anti-Y 29/55 with bone marrow cells from children with ALL at presentation and in remission was studied.

MATERIALS AND METHODS

Mononuclear bone marrow cells of children were separated by Hypaque-Ficoll (Seromed, Munich, West Germany) density gradient centrifugation and processed as described previously (6). The following monoclonal antibodies were used to characterize leukemic and normal cells in suspension: anti-CALLA and anti-PT (both kindly provided by Dr. S. Carrel, Ludwig Institute for Cancer Research, Epalinges/Lausanne, Switzerland); anti-human HLA-DR (la-like antigens), anti-Leu-1 (both from Becton Dickinson, Sunnyvale, Calif.), and anti-Y 29/55 (3). All monoclonal antibodies were used in an indirect immunofluorescence assay, the second antibody being either a FITC- or a TRITC-labeled goat anti-mouse immunoglobulin (Nordic Laboratories, Tilburg, The Netherlands), or a TRITC-labeled Fab fragment of goat anti-mouse IgG (Cappel Laboratories, Cochranville, Pa.). In addition, a TRITC-labeled Fab fragment of goat anti-human IgM (Cappel Laboratories) was applied. Nuclear TdT was assessed with an immunofluorescent assay kit (Bethesda Research Laboratories, Rockville, Md.) as described (7). By means of these reagents, ALL was subdivided into common ALL (CALLA positive, la positive, TdT positive), null ALL (CALLA negative, la positive, TdT positive), pre-B-ALL (CALLA positive, la positive, TdT positive, clgM positive), pre-B-B-ALL (CALLA positive, la positive, TdT positive, clgM positive, slgM positive or negative), and T-ALL (PT positive, Leu-1 positive). The definition of T-ALL by 2 PT antibodies was chosen since not all cases of T-ALL are erythrocyte rosette positive. There were no patients with B-ALL.

For the combined assessment of the Y 29/55 antigen and of clgM, bone marrow cells were incubated first with anti-Y 29/55 followed by goat anti-mouse immunoglobulin-FITC. Cells were pelleted then onto glass slides in a cytocentrifuge, fixed for 20 min in ice-cold ethanol supplemented with 5% glacial acetic acid, rinsed in 0.1 M phosphate buffered saline, and air-dried. Finally, glass slides were incubated with goat anti-mouse IgM followed by anti-goat IgG-FITC and TRITC-labeled goat anti-mouse IgM.
After extensive rinsing in 0.1 M phosphate-buffered saline and mounting of KH2PO4 in deionized water to 1000 ml), and stained with a F(ab')2 fragment of goat anti-human IgM-TRITC for 30 min at room temperature. After extensive rinsing in 0.1 M phosphate-buffered saline and mounting with Fluoromount (Hopkin & Williams, Chadwell Heath, Essex, England), slides were evaluated for fluorescence.

For kinetic investigations (determination of labeling index), freshly drawn bone marrow was incubated in vitro for 30 min, at 37°, with 4 μCi of [3H]dThd (New England Nuclear, Dreieich, West Germany; specific activity, 6.7 Ci/mmol) per ml of bone marrow. Mononuclear cells were then separated by Hypaque-Ficoll density centrifugation, processed for immunofluorescence, and fixed on microscope slides as described above. Slides were mounted on a computer-controlled scanning table, and fluorescence-positive cells could then be counted by use of a PDP-12 computer (digital) programmed with Apamos II (Carl Zeiss, Oberkochen, West Germany). Provisional morphological evaluation was done by Nomarski interference and by conventional staining.

RESULTS

Anti-Y 29/55 and slgM marker analyses of 14 patients with common ALL, 4 patients with pre-B-ALL, 2 patients with pre-B-B-ALL, 4 patients with null-cell ALL, and 3 patients with T-ALL are summarized in Table 1. In 26 of 27 patients, a maximum of 2% of anti-Y 29/55-reacting cells were found. Morphologically, these reacting cells were small lymphocytes and probably represented residual normal B-lymphocytes. One patient with pre-B-B-ALL had 11% anti-Y 29/55-positive cells. In size distribution and morphology, these cells resembled the anti-Y 29/55-negative leukemia cells. In this patient, 62% of the leukemic cells were slgM positive, and 10% exhibited faint slgM, thus characterizing this ALL as a transitional pre-B-B-ALL. The percentages of anti-Y 29/55-positive and of slgM-positive cells were nearly equal, but since no simultaneous incubation was done, we do not know whether both markers were present on the same cell or not. The other patient with pre-B-B-ALL had 21% of slgM-positive but practically no anti-Y 29/55-positive cells.

For investigation of normal B-cell precursors, lymphoid cells in the bone marrow of one child with chronic neutropenia and of 5 children with acute leukemia in remission shortly after cessation of long-term chemotherapy were studied. At this stage, a relative lymphocytosis is observed (1, 10). The percentage of anti-Y 29/55-positive cells varied between 1.4 and 8.7. Approximately 60 to 90% of anti-Y 29/55-positive cells were also strongly slgM positive. However, extremely few slgM-positive, anti-Y 29/55-negative cells were seen. Morphologically, anti-Y 29/55-positive cells corresponded to small lymphocytes.

Bone marrow cells of 2 of the aforementioned patients with relative lymphocytosis were stained in suspension for anti-Y 29/55 (FITC), fixed on slides, and stained for cytoplasmic IgM (TRITC). The cells were then assessed simultaneously for FITC and TRITC fluorescence. As shown in Table 2, anti-Y 29/55-negative, slgM-positive cells accounted for 0.6% of all mononuclear cells in one patient and 2.5% in the other patient. Morphologically, these cells were small to large lymphoid elements with a narrow rim of cytoplasm. The labeling index of these cells was 27.8% in one and 23.8% in the other patient. Anti-Y 29/55-positive, slgM-positive or negative cells accounted for 5.6% of all mononuclear cells in one patient and 1.5% of all mononuclear cells in the other patient. Morphologically, these cells corresponded to small lymphocytes. None were labeled after [3H]-dThd pulse.

DISCUSSION

The monoclonal antibody anti-Y 29/55 recognizes human leukemia B-lymphocytes in various states of differentiation and normal B-lymphocytes found in secondary lymphoid organs like lymph nodes, spleen, and tonsils (3). It is not known, however, at which level of B-cell differentiation the Y 29/55 antigen appears. Since approximately 20% of the children with non-T-ALL have pre-B-ALL and since some of the latter even have leukemic cells with both cytoplasmic and surface IgM (pre-B-B-ALL) (2, 4, 11, 12), it was of interest to study the reaction of ALL cells with the anti-Y 29/55 antibody.

Our results (Table 1) demonstrate that neither common, nor null or T, nor pre-B-ALL cells react with this antibody. It is noteworthy, however, that in one of 2 patients with intermediate pre-B-B-ALL 11% anti-Y 29/55-positive leukemic cells were found. This observation suggests that the reactivity of the anti-Y 29/55 antibody is related to the presence of surface immunoglobulin. It suggests, furthermore, that the leukemic cells in patients with pre-B-ALL could be arrested at slightly different levels of differentiation.

The investigations on patients with relative lymphocytosis in the bone marrow were done in order to study the anti-Y 29/55 reactivity of normal B-cell precursors. The observation that in suspension 60 to 90% of anti-Y 29/55-positive cells were strongly slgM positive and that practically no slgM-positive cells were anti-Y 29/55 negative suggests that normal lymphoid bone marrow cells with surface immunoglobulin in children are anti-Y 29/55 positive and that slgM-negative, anti-Y 29/55-positive cells might have surface immunoglobulin with other heavy chains.

Table 1
Reactivity of monoclonal anti-Y 29/55 and anti-IgM with various subtypes of ALL in children

<table>
<thead>
<tr>
<th>Subtype of ALL</th>
<th>No. of patients</th>
<th>Diagnostic marker</th>
<th>Median % of cells with diagnostic marker</th>
<th>Median % of Y 29/55-positive cells</th>
<th>Median % of slgM-positive cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common</td>
<td>14</td>
<td>CALLA</td>
<td>55 (28-95)</td>
<td>0.5 (0-2)</td>
<td>0.75 (0-2.5)</td>
</tr>
<tr>
<td>Pre-B</td>
<td>4</td>
<td>slgM</td>
<td>70 (65-86)</td>
<td>0.5 (0-2)</td>
<td>0.5 (0-2)</td>
</tr>
<tr>
<td>Pre-B-B</td>
<td>2</td>
<td>slgM</td>
<td>62</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>slgM</td>
<td>34</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>Null</td>
<td>4</td>
<td>CALLA</td>
<td>1.5 (0-5)</td>
<td>1 (0.5-2)</td>
<td>1.25 (0.5-4)</td>
</tr>
<tr>
<td>T</td>
<td>3</td>
<td>PT</td>
<td>88 (55-95)</td>
<td>0.5 (0-0.5)</td>
<td>0.5 (0-1)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, range.
Table 2
Combined determination of anti-Y 29/55 (FITC), intracytoplasmic IgM (TRITC), and in vitro [3H]dThd incorporation in bone marrow cells of 2 children with relative lymphocytosis

<table>
<thead>
<tr>
<th>Marker pattern</th>
<th>% of mononuclear cells</th>
<th>Labeling index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Y 29/55-negative, clgM-positive</td>
<td>0.6</td>
<td>27.8</td>
</tr>
<tr>
<td>Anti-Y 29/55-positive, clgM-positive</td>
<td>5.3</td>
<td>0</td>
</tr>
<tr>
<td>Anti-Y 29/55-positive, clgM-negative</td>
<td>0.3</td>
<td>0</td>
</tr>
<tr>
<td>Case 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Y 29/55-negative, clgM-positive</td>
<td>2.5</td>
<td>23.8</td>
</tr>
<tr>
<td>Anti-Y 29/55-positive, clgM-positive</td>
<td>0.3</td>
<td>0</td>
</tr>
<tr>
<td>Anti-Y 29/55-positive, clgM-negative</td>
<td>1.5</td>
<td>0</td>
</tr>
</tbody>
</table>

The results shown in Table 2 indicate that a small fraction of clgM-positive cells with a high [3H]dThd labeling index was anti-Y 29/55 negative. Since lack of anti-Y 29/55 reactivity practically excluded the presence of slgM, this population was considered to be anti-Y 29/55-negative, normal pre-B-cells. Although the presence of anti-Y 29/55-positive, clgM-positive cells might suggest that not all normal pre-B cells are anti-Y 29/55 negative, one must consider the possibility that, under the experimental conditions chosen, slgM may have reacted while clgM was assessed as suggested by Pearl et al. (9). The fact that these anti-Y 29/55-positive, clgM-positive cells were small lymphocytes not labeled after a [3H]dThd pulse also favors this interpretation, since practically all normal B-cells in bone marrow remain unlabeled after a [3H]dThd pulse (data not shown).

In conclusion, our results suggest that the anti-Y 29/55 reactivity in leukemic and normal B-cell precursors is related to the appearance of surface immunoglobulins.

ACKNOWLEDGMENTS
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REFERENCES
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