mb-1: A New Marker for B-Lineage Lymphoblastic Leukemia

By Valeria Buccheri, Biljana Mihaijević, Estela Matutes, Martin J.S. Dyer, David Y. Mason, and Daniel Catovsky

The expression of the Ig-linked mb-1 polypeptide was analyzed by immunocytochemistry (alkaline phosphatase anti-alkaline phosphatase technique) using a specific monoclonal antibody in 165 cases of acute leukemia, with 88 being lymphoblastic (ALL) and 77 myeloid (AML). The purpose of the study was to investigate the specificity of this reagent for B-lineage cases and its reactivity on leukemias that coexpress myeloid and B-cell antigens (biphenotypic). The majority (89%) of 72 B-cell precursor ALL patients were positive. Of these, mb-1 was expressed in all 9 patients with early-B-ALL (CD10+), in all 11 patients with pre-B-ALL (smlgM+), and in the single case of B-ALL (smlgM+). Forty-three of 51 patients with common-ALL (CD10+, smlgM+) were also positive. All 16 T-lineage ALL patients and 72 (93.5%) of the AML patients examined were mb-1 negative. Four of the 5 mb-1-positive AML patients were considered biphenotypic and expressed other B-cell antigens such as CD10, CD19, and/or cCD22 and all showed rearrangement of the Ig heavy chain genes. Within the AML cases, mb-1 and cCD22 were more useful than other B-cell antigens in detecting biphenotypic cases, and mb-1 showed the highest correlation with the clonal rearrangement of Ig heavy chain genes. These results indicate that mb-1 is a sensitive and specific reagent for B-lineage blasts that will aid in the classification of B-cell precursor ALL and in the identification of biphenotypic leukemia presenting as AML.

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MEMBRANE IgS (smlg) and T-cell receptor (TCR) molecules constitute, respectively, the B- and T-cell antigen receptors that give the immune system the capacity to identify specific antigens. The expression of Ig and TCR molecules is acquired during lymphoid differentiation through unique rearrangement of the Ig or TCR genes, which differ in each lymphocyte.1 2

IgM and IgD are the major Ig classes on the membrane of circulating B lymphocytes. Both are noncovalently associated on human B cells with at least two structurally distinct membrane glycoproteins designated α and β with molecular size of 47 and 37 Kd, respectively.3 The 47-Kd molecule is encoded by the human homologue of the murine mb-1 gene4 partially cloned by Sakaguchi et al5 and by Yu and Chang.6 The 37-Kd molecule is the product of a different gene and is encoded by the B29 gene.6 7 8 No structural relationship between the isolated 47-Kd heterodimer and B-cell antigens such as CD24, CD37, or CD72 exists6; therefore, the mb-1 protein product is a new human B-cell antigen that is also present in B cells expressing other smlg isotypes.6 9

Antibodies have recently been raised against the human mb-1 protein and were shown to be B-cell specific.10 The mb-1 protein was found to appear early during B-cell differentiation preceding cytoplasmic μ chains. Therefore, it is of potential value for detecting B-cell-precursor ALL. Here, we have investigated the specificity and diagnostic role of an anti-mb-1 antibody in a large series of acute leukemia cases. In particular, we have focused on biphenotypic cases that coexpress myeloid and lymphoid antigens to assess whether this reagent could help provide evidence for B-lineage differentiation and, consequently, in the classification of this disease group.

MATERIALS AND METHODS

Material from 165 consecutive patients with acute leukemia was investigated. Most cases were de novo leukemias, and the samples were obtained at the time of diagnosis. Twenty-three percent of the cases were studied during a relapse. The majority of peripheral blood or bone marrow samples had more than 70% blast cells.

Based on the morphologic and cytochemical criteria of the French-American-British Cooperative Group, 88 were classified as ALL and 77 as AML.11 13 In the AML group the age ranged from 2 to 91 years (median 43), whereas in ALL the age ranged from 1 month to 81 years (median 13). Ten AML and 47 ALL patients were children (≤15 years). In addition, AML and ALL cases with unexpected expression of several lymphoid or myeloid antigens, respectively, were designated biphenotypic.14 Overall, 16 cases (11 AML and 5 ALL) were reclassified as biphenotypic.

Monoclonal antibodies (MoAbs). To assess membrane and cytoplasmic antigens, immunophenotyping was performed with a panel of MoAbs consisting of antiamyloid and antilymphoid (B and T) reagents (Table 1). The nuclear enzyme TdT was evaluated with a polyclonal rabbit antisemur (SeraLab, Crawley Down, UK). All cases were tested with the MoAb HM-57 against the human mb-1 polypeptide, as described previously. MoAb HM-57 was produced by immunizing BALB/c mice with a synthetic peptide established from the human mb-1 carboxyl-terminal sequence.10

Immunophenotyping. Mononuclear cells from peripheral blood and/or bone marrow samples were isolated on a Lymphoprep (Nyegaard, Oslo, Norway) density gradient centrifugation. Cytospin slides and cell smear preparations were air dried overnight, wrapped in aluminum foil, and stored at −20°C.

Surface antigens were detected using a FACScan (Becton Dickinson, San Jose, CA) flow cytometer. Viable cells were incubated either directly with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) MoAb or indirectly with unlabeled murine MoAb and a second layer of FITC conjugated IgG goat anti-mouse–Ig (Dynatech, Billingshurst, UK). All incubations were performed for 30 minutes at 4°C. Double labeling was performed on a single step by
incubating the cells directly with FITC- and PE-conjugated MoAb of different Ig isotypes. Appropriate controls for the single and double staining were set up by replacing the relevant MoAb with a mouse Ig of the same Ig isotype and/or a mixture of mouse Ig of different Ig isotypes. Results were considered positive for all MoAbs if more than 20% of the cells were reactive in excess of the negative control in the blast-cell region gated.

Immunocytochemistry was used for labeling intracytoplasmic antigens (mβ-1, cIgM, cCD22, cCD3, and myeloperoxidase [MPO]) and the nuclear enzyme TdT on cytocentrifuged, fixed cells, using standard alkaline phosphatase anti-alkaline phosphatase method. 19 The reactivity with these MoAbs was seen as a distinct red deposit. With this method, blast cells were easily distinguished from normal cells by light microscopy. For this reason, hematopoietic blast staining were set up by replacing the relevant MoAb with a mouse Ig of the same Ig isotype and/or a mixture of mouse Ig of the opposite charge (E+). 16,17 The reactivity with these MoAbs was assessed using Fisher's exact test. A two-sided P value of .05 was considered to be of statistical significance. 19

RESULTS

ALL. 88 ALL cases were MPO negative by light microscopy cytochemistry. Immunologic analysis of ALL showed that 82% were of B-cell lineage and that 18% were of T-cell lineage. The reactivity of the 72 cases of B-cell-lineage ALL with the various B-lymphoid–associated antigens showed that 9 cases (14%) corresponded to early B-ALL (CD19+, CD10–, IgM+), 11 (16%) to common-ALL (CD19+, CD10+, cμ+), and 11 (15%) to pre-B-ALL (cμ+, CD10+, CD19+), and 1 (1%) to B-ALL (smIgM+, CD10–, CD19+).20

The reactivity with anti-mβ-1 was evaluated in B-cell precursor ALL in relation to the expression of the other B-cell antigens (Table 2). Overall, mβ-1 was expressed in the great majority of cases. Of these, all early B-cell ALL and 43 of 51 common-ALL were positive. mβ-1 positivity was detected in all cases of pre-B-ALL and B-ALL (Fig 1). In these cases, the proportion of cells reactive with mβ-1 and the intensity of the staining were similar to those of the cytoplasmic μ chain, except in 2 pre-B-ALL cases in which only 11% and 16% of blasts were mβ-1 positive. Except for those 2 cases, the mean percentage of mβ-1-positive blasts was 55.8% (range, 20% to 94%). The proportion of mβ-1-positive cases was comparable with those for CD19 and CD22 (Table 2).

Molecular analysis performed in all 25 B-lineage ALL cases, including the 4 reclassified as biphenotypic, showed rearrangements or deletions of the Ig heavy chain genes. There were also rearrangements of the TCR δ, γ, and β gene in 78%, 59%, and 39% of the cases, respectively.

Table 2. Distribution of 72 B-Cell Precursor ALL Cases According to Immunophenotype

<table>
<thead>
<tr>
<th>Cases</th>
<th>CD10</th>
<th>CD19</th>
<th>CD22</th>
<th>mb-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early-B-ALL</td>
<td>9</td>
<td>9</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Common-ALL</td>
<td>51</td>
<td>51</td>
<td>46*</td>
<td>46*</td>
</tr>
<tr>
<td>Pre-B-ALL</td>
<td>11</td>
<td>7</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>B-ALL</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>58</td>
<td>66</td>
<td>66</td>
</tr>
</tbody>
</table>

Note: The blast cells in all cases expressed class II antigens. All but one (B-ALL) were TdT positive. Considering the positive cases the mean percentage of blast cells expressing CD10, CD19, and CD22 were 79%, 81%, and 70.4%, respectively. Five cases reclassified as biphenotypic because they expressed 2 or more myeloid antigens are included in this analysis. Percentages are in parentheses. * Forty-nine cases tested.
AML. All AML cases but one expressed at least one myeloid-associated antigen (CD13, CD14, CD33, and anti-MPO). Overall, CD13 and/or CD33 were positive in 97.3% of the cases. The mean percentage of blasts expressing CD13 was 64% (range, 30% to 99%) and for CD33 was 70% (range, 26% to 99%).

Five cases were mb-1 positive (Fig 2). Of these, only 1 (AML-M2) did not express other lymphoid antigens and showed 18% mb-1-positive blasts. This was the only case (1.5%) of 66 typical AML cases (not considered biphenotypic) that was mb-1 positive. The remaining 4 mb-1-positive AML cases (28%, 37%, 39%, and 66% positive blasts) were reclassified as biphenotypic because they expressed 2 or more lymphoid antigens (CD10+/CD19+ in 2 and CD19+/cCD22+ in other 2). All were TdT positive and had more than 80% of blasts in the bone marrow. Double marker studies with CD19 and CD13 or CD33 clearly showed coexpression of lymphoid and myeloid antigens on the same leukemic cells. The percentage of cells coexpressing CD19+ and CD13+ was 58% and 70%, respectively, in 2 cases and 21% and 44%, respectively, in the other 2.

The expression of mb-1 was compared with that of other
Table 3. Correlation Between Expression of B-Cell Antigens in AML and Presence of Ig Gene Rearrangements

<table>
<thead>
<tr>
<th>MoAbs</th>
<th>No. of Positive Cases (n = 77)</th>
<th>No. of Cases B lymphoid antigen*</th>
<th>No. of Positive Cases (n = 38)</th>
<th>Ig Gene Rearrangements</th>
<th>p Value\dagger</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD10</td>
<td>9 (12)</td>
<td>4</td>
<td>6</td>
<td>3 (60)</td>
<td>.04</td>
</tr>
<tr>
<td>CD19</td>
<td>8 (10)</td>
<td>4</td>
<td>6</td>
<td>4 (66)</td>
<td>.03</td>
</tr>
<tr>
<td>cCD22</td>
<td>6 (8)</td>
<td>6</td>
<td>6</td>
<td>3 (50)</td>
<td>.04</td>
</tr>
<tr>
<td>mb-1</td>
<td>5 (6)</td>
<td>4</td>
<td>5</td>
<td>4 (100)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Percentages are in parentheses.
* By expressing two or more lymphoid antigens.\(^\dagger\)
\dagger Fisher’s exact test.

B-lymphoid antigens in the 77 cases of AML (Table 3). Although not statistically significant, mb-1 and cCD22 were less frequently expressed and were positive almost exclusively in cases classified as bipheno-

typic. Ig and/or TCR gene rearrangements were investigated in 38 cases of AML. Five patients (13%) had Ig heavy and/or light chain gene rearrangements, and of these, 4 corresponded to the mb-1-positive cases reclassified as bipheno-
typic (Table 3). The remaining case (AML-M4) did not express lymphoid antigens and showed clonal rearrangement of the Ig heavy and \(\kappa\) light chain. Of the 6 other AML cases considered as bipheno-
typic, only 1 had Ig gene rearrangements, but mb-1 was negative. Ig gene rearrangement was not studied in the single case with isolated expression of mb-1.

When we examined the correlation between expression of individual B-cell antigens in AML and Ig gene rearrangement, the correlation of mb-1 with Ig gene rearrangement was the most significant (Table 3).

DISCUSSION

This study of a large series of acute leukemias showed that mb-1 is specifically associated with B-cell-lineage ALL cases and with bipheno-
typic leukemia cases that express myeloid and B-cell antigens and have Ig heavy chain genes in rearranged configuration. Therefore, it is likely that anti-
mb-1 antibodies will become useful for the further characterization of blast cells, particularly in the context of AML with bipheno-
typic features as other B- and T-cell antigens are less consistently associated with Ig gene rearrangements than the expression of mb-1 (Table 3).

Our results showed that 89% of B-cell precursor ALL cases were positive for mb-1. In agreement with the original report by Mason et al.,\(^\text{10}\) our study suggests that mb-1, although linked to IgM in mature B cells, is expressed before cytoplasmic \(\mu\) chain during B-cell differentiation because it could be detected in the absence of cytoplasmic \(\mu\) chain. The few negative mb-1 cases were found in common-ALL, but, at present, we do not know if this represents a distinct subgroup.

This analysis has also shown that mb-1 provides additional evidence for B-lymphoid commitment in cases of bi-

phenotypic leukemia, strengthening the biologic definition of these cases as a distinct clinicopathologic entity. It is currently accepted that the concurrent expression of anti-
gens from different lineages is a feature of a proportion of ALL and AML cases. Data from the literature show that lymphoid-associated antigens can be detected in up to 60% of cases with typical features of AML, although the coex-
pression of several of these markers is found only in a minority.\(^\text{21-24}\) Ig and/or TCR gene rearrangements are also documented in approximately 15% of AML cases,\(^\text{25}\) but there is little information on the correlation between the expression of lymphoid antigens and the incidence of gene rearrange-
ments in such cases. In our series of AML,\(^\text{14}\) 12% of cases showed Ig and/or TCR gene rearrangement, and there was a statistically significant correlation with the presence of sev-
eral B-lymphoid antigens in such cases.

mb-1 was positive in 6% of AML cases, but 4 of the 5 positive cases also expressed 2 other B-cell antigens and were reclassified as bipheno-
typic.\(^\text{14}\) The 4 cases showed rearrangement of the Ig genes, providing additional evidence for the lymphoid commitment of these cells. mb-1 was only positive in 1.5% of AML without bipheno-
typic features. When the positivity for each B-cell antigen in AML was studied separately and compared with the findings obtained from molecular analysis, there was a statistically significant correlation with the presence of mb-1 (Table 3). Although more cases need to be studied, our findings show that chances are good for identifying cases of AML with Ig gene rearrangements when mb-1 is positive.

Immunologic marker analysis of leukemic cells is an important tool for diagnostic and research purposes and has improved continuously through the development of new lineage-specific antibodies. We suggest that mb-1 should be incorporated into the routine immunophenotyping of acute leukemia as a marker of B-lineage blasts in ALL and of bipheno-
typic leukemia presenting as AML.

ACKNOWLEDGMENT

We thank Dr T.H. Rabbitts (MRC Laboratory of Molecular Biology, Cambridge) for the gift of the Ig and TCR probes.

REFERENCES

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