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

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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+, cdμ+) in all 11 patients with pre-B-ALL (cdμ+) and in the single case of B-ALL (smIgM+). Forty-three of 51 patients with common-ALL (CD10+, cdμ+) 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 cdCD22 and all showed rearrangement of the Ig heavy chain genes. Within the AML cases, mb-1 and cdCD22 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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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 plc, Amersham, & Polyclonal antibody.

Southern blot analysis. Molecular analysis, which included evaluation of Ig and TCR gene rearrangement, was performed in 34 ALL (25 B-cell lineage and 9 T-ALL) and 38 AML cases. High molecular weight DNA extracted from mononuclear cells was digested with BamHI, EcoRI, and HindIII (Stratagene, La Jolla, CA; Gibco BRL, Luttenvorth, UK) restriction enzymes, size fractioned by electrophoresis on 0.6% to 0.8% agarose gel, and transferred to positively charged nylon filters (Hybond N+; Amersham, UK). Hybridization conditions according to standard procedures and exposed to X-ray films (Fuji Photo Film Co, Japan) in the presence of intensifying screens for 1 to 5 days at ~80°C.

Ig heavy and light chain DNA probes included IgH probe (clone M13C6R51A), a 2.5-kb EcoRI-Bgl II genomic fragment containing the joining region of the IgH locus; Igκ probe (clone λC75), a 1.2-kb EcoRI genomic fragment subcloned into pUC9; Igκ probe (clone cP3A3), a 700-bp Bgl II-EcoRI genomic fragment subcloned in pUC. The TCR genes were analyzed using TCR Cβ1 probe (clone M131B08B1), a 700-bp EcoRI-HindIII genomic fragment; TCR Jγ1 probe (clone M13H60), a 700-bp EcoRI-HindIII genomic fragment; TCR Jβ1 probe (clone J61.6), a 1.6-kb Sac I fragment of the DNA region 3 to the Jβ1; and TCR Cδ probe (clone R21EE), a 3.5-kb EcoRI fragment.

Statistical analysis. Differences between subgroups of patients were assessed using Fisher’s exact test. A two-sided P value of .05 was considered to be of statistical significance.

RESULTS

ALL. 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-, CD19+), 11 (69%) to common-ALL (CD19+, CD10+, CD19+), 11 (15%) to pre-B ALL (CD10+, CD10+, CD19+), and 1 (1%) to B-ALL (smIgM+, CD10+, CD19+). The reactivity with anti-mb-1 was evaluated in B-cell precursor ALL in relation to the expression of the other B-cell antigens (Table 2). Overall, mb-1 was expressed in the great majority of cases. Of these, all early B-cell ALL and 43 of 51 common-ALL were positive. mb-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 mb-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 mb-1 positive. Except for those 2 cases, the mean percentage of mb-1-positive blasts was 55.8% (range, 20% to 94%). The proportion of mb-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 |
|--------------------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| No. of Cases | CD10 | CD19 | CD22 | mb-1 |
| Early-B-ALL | 9 | 9 | 9 | 9 |
| Common-ALL | 51 | 51 | 46* | 46* | 43 |
| Pre-B-ALL | 11 | 7 | 10 | 11 | 11 |
| B-ALL | 1 | 1 | 1 | 1 |
| Total | 72 | 58 | 66 | 66 | 64 |

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
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 biphenotypic.

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 biphenotypic (Table 3). The remaining case (AML-M4) did not express lymphoid antigens and showed clonal rearrangement of the Ig heavy and κ light chain. Of the 6 other AML cases considered as biphenotypic, 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 biphenotypic 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 biphenotypic 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.,10 our study suggests that mb-1, although linked to IgM in mature B cells, is expressed before cytoplasmic μ chain during B-cell differentiation because it could be detected in the absence of cytoplasmic μ 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 biphenotypic leukemia, strengthening the biologic definition of these cases as a distinct clinicopathologic entity. It is currently accepted that the concurrent expression of antigens 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 coexpression of several of these markers is found only in a minority.21-24 Ig and/or TCR gene rearrangements are also documented in approximately 15% of AML cases,25 but there is little information on the correlation between the expression of lymphoid antigens and the incidence of gene rearrangements in such cases. In our series of AML,14 12% of cases showed Ig and/or TCR gene rearrangement, and there was a statistically significant correlation with the presence of several 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 biphenotypic.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 biphenotypic 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 biphenotypic leukemia presenting as AML.

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