Lack of CD45 Antigen on Blast Cells in Childhood Acute Lymphoblastic Leukemia Is Associated With Chromosomal Hyperdiploidy and Other Favorable Prognostic Features

By Fred G. Behm, Susana C. Raimondi, Michael J. Schell, A. Thomas Look, Gaston K. Rivera, and Ching-Hon Pui

The leukocyte common antigen (CD45) was detected on the surface of leukemic cells in 217 (87%) of 249 cases of newly diagnosed childhood acute lymphoblastic leukemia (ALL). All 55 cases of T-lineage ALL, compared with 159 of 191 B-lineage cases, expressed the CD45 antigen (P = .0005). The frequency of CD45 expression did not differ between cases of early pre-B (CD19+, cytoplasmic μ−) and pre-B (CD19+cytoplasmic μ+) ALL. Cases of ALL lacking CD45 had significantly lower leukocyte counts (P = .002) and serum lactate dehydrogenase (LDH) levels (P = .007) and were more likely to have leukemic cell hyperdiploidy greater than 50 (P < .0001) or a DNA index greater than 1.15 (P < .0001), as compared with cases positive for the antigen. Of the 130 patients whose follow-up duration was sufficient for analysis of event-free survival, the 53 with the highest levels of CD45 expression (>90%) were the most likely to have an adverse event on intensive multiagent chemotherapy. Patients without detectable CD45 had a negligible risk of failure. This study suggests a relationship between the expression of the CD45 antigen on leukemic lymphoblasts and other biologic factors that influence prognosis in ALL.

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The leukocyte common antigen, or CD45, is found on all hematopoietic cells except erythrocytes and platelets, and is absent from nonhematopoietic tissues.1-3 CD45 antigens belong to a family of cell surface glycoproteins with a heavily glycosylated extracellular domain, a transmembrane segment, and a large cytoplasmic domain that contains tyrosine phosphatase activity.1,4 These glycoproteins are encoded by a single gene located in a syntenic region of chromosome 1q32 in humans.5 CD45 appears in at least four discrete isoforms that differ in carbohydrate and protein backbone structure.6,7 Data published by the Third and Fourth International Workshops on Human Leukocyte Differentiation Antigens and by others demonstrate that anti-CD45 antibodies belong to two major clusters: those that detect a framework structure common to all members of the family, and restricted groups (CD45RA, CD45RB, and CD45RO) that recognize discrete epitopes within the variable N terminus of the exterior portion of the molecule.8-12 Recent findings suggest that CD45 may be involved in regulation of hematopoietic cell growth, differentiation, and cell activation.13,17

Despite extensive study of CD45 glycoproteins, the frequency of their expression and potential clinical significance in leukemia and lymphoma remain poorly defined. By analyzing a large number of consecutive cases of newly diagnosed acute lymphoblastic leukemia (ALL), we found that CD45 positivity is related to adverse prognostic features, both clinical and biologic. Patients with undetectable CD45 expression on their leukemic blasts had the most favorable presentation and the highest probability of continuous event-free survival.

MATERIALS AND METHODS

Patients. From November 1986 to September 1990, 258 consecutive children with newly diagnosed ALL were admitted to St. Jude Children's Research Hospital and enrolled in one of two total therapy studies (XI and XII). Complete immunophenotyping and cytogenetic analyses were performed on leukemic blast cells from 249 of these patients. Follow-up times were adequate for prognostic determinations only in study XI (n = 130), which tested the efficacy of six-drug induction/consolidation therapy and continuation treatment with rapidly rotated drug pairs.18 Informed consent was obtained from all patients or their guardians, and the investigations were approved by the institution's clinical trials review committee.

Morphologic and cytochemical studies. Bone marrow cells were stained by standard techniques, including Wright-Giemsa, myeloperoxidase, Sudan Black B, naphthol AS-D chloroacetate esterase, and α-naphthyl butyrate esterase. The diagnosis of ALL was based on morphologic and cytochemical criteria of the French-American-British (FAB) Cooperative Working Group.10,19 Thus, by definition, all cases had fewer than 3% myeloperoxidase-positive blasts.

Immunophenotyping. Bone marrow cells were separated on a Ficoll-sodium metrizoate gradient, and only samples containing greater than 85% blasts were assayed. Cell surface antigens were detected by a standard indirect immunofluorescence method using a panel of monoclonal antibodies that reacted with CD1 (Leu-6), CD2 (T11), CD3 (Leu-4), CD4 (Leu-3a), CD5 (Leu-1), CD7 (Leu-9), CD8 (Leu-2a), CD10 (J5), CD13 (MY7), CD14 (MY4 and Leu-M3), CD15 (MY1), CD19 (Leu-12), CD20 (Leu-16), CD21 (anti-CR2), CD22 (Leu-14), CD33 (MY9), CD34 (HPCA-1/MY10), CD36 (5F1), CD41 (α-gpIIb/IIIa), CD45 (HLe-1/2D1), CD71 (antitransferrin receptor), and anti-HLA-DR. In all experiments, isotype-matched murine myeloma immunoglobulins and anti-β2-microglobulin were used as negative and positive controls, at the same protein concentrations as the test antibodies. Leukemic cells were also tested for surface and cytoplasmic immunoglobulins (sIgM and cIgM) with goat anti-human μ (Southern Biotechnology, Birmingham, AL). An indirect immunofluorescent technique was used to detect nuclear terminal deoxynucleotidyl transferase (Tdt).

Fluorescence activity was analyzed by an EPICS C flow cytometer equipped with a 5-W Coherent laser (Coulter, Hialeah, FL) for
monoclonal antibody studies or a Zeiss epifluorescent microscope for clgα, slgα, and Tdt analyses. Histograms of fluorescent intensity were based on a log scale. The percentage of positive cells was obtained by counting the number of events with fluorescent intensity greater than the isotype-matched negative control.

Leukemic samples were considered positive for a particular antigen if greater than or equal to 20% of leukemic cells reacted with a particular monoclonal antibody or if greater than or equal to 10% were positive for CD34, clgα, and Tdt. The results of CD45 assays in B-lineage ALL were corrected for contaminating normal T cells in the test sample using the formula \([(%\text{CD}45 - %\text{CD}3)/ (100 - %\text{CD}3) \times 100]\). Because all samples of T-lineage ALL contained greater than 90% blasts, no correction for contaminating normal hematopoietic cells was made. Immunophenotyping data were used to classify cases as T cell (CD7' plus CD5+ or CD2+), B cell (slgα'), pre-B (clgα'), or early pre-B (CD19', CD22', HLA-DR', CD7', CD5', clgα', slgα', and CD10').

Cytogenetic and DNA flow cytometric studies. Immediately after collection, bone marrow specimens were processed for chromosomal analysis by the direct method of Williams et al.22 Metaphase preparations were G-banded with trypsin and Wright's stain. Chromosome abnormalities were classified according to the International System for Human Cytogenetic Nomenclature.23 A sample was considered to be hyperdiploid if two or more metaphases had a modal chromosome number of 51 or more. Leukemic marrow samples were also stained with propidium iodide and analyzed for cellular DNA content by flow cytometry as previously described.24 The results were expressed as the DNA index (ratio of DNA content in leukemic G0/G1 cells vs normal diploid G0/G1 cells), a measure that correlates closely with chromosome number. Thus, the DNA index of diploid cells is 1.00 and that of cells with more than 53 chromosomes is greater than 1.15.

Statistical analysis. Differences in the distribution of clinical and biological features among groups of patients were tested by the two-tailed Fisher's exact test for two-by-two tables or the Pearson \(\chi^2\) test. The Wilcoxon rank-sum test was used to compare the percentage of CD45 expression on leukemic blasts of T-cell and B-lineage ALL cases. The correlation between the percentage of CD45+ blasts and that of other markers was examined using Spearman's rank correlation. Plots were made using the monotone smoothing algorithm with a running mean smoother.24 Event-free survival curves were constructed by the Kaplan-Meier procedure,25 with differences analyzed by the log-rank and trend tests. Early death or failure to enter remission was considered an event at zero time. The effects of potentially independent prognostic factors (age, sex, race, leukocyte count, serum lactic dehydrogenase [LDH], CD45 expression, DNA index, leukemic cell ploidy) on event-free survival was tested by the Cox proportional hazards model. A stepwise selection procedure was used to determine factors that were significantly related to event-free survival. Analysis of treatment outcome was limited to 130 consecutive patients who were treated in study XI, as the duration of follow-up for patients in study XII remains relatively short.

RESULTS

CD45 expression was detected in 217 (87%) of the 249 cases of childhood ALL. All 55 T-cell cases, compared with 159 of the 191 cases of B-lineage ALL, expressed CD45 (\(P = .0005\)). Indeed, expression of the antigen was more extensive for T-cell ALL than for B-lineage ALL (\(P = .0003\), Fig 1). The pattern of CD45 expression did not differ between cases of early pre-B and pre-B ALL. No correlations were identified between levels of expression of CD45 and CD10, CD19, CD21, CD22, CD13, CD14, CD33, CD71, or Tdt. However, CD45 expression was inversely related to that of the CD20 antigen (\(r = -.21, P = .002\)) and the CD34 antigen (\(r = -.15, P = .04\)).

Table 1 summarizes selected presenting clinical and biological features among groups of patients with CD45 expression levels in T-cell and B-lineage ALL.

<table>
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<tr>
<th>WBC count (X 10⁹/L)</th>
<th>&lt;= CD45+ Blasts</th>
<th>&gt;= CD45+ Blasts</th>
<th>CD45+ vs CD45-</th>
<th>CD45+ vs CD45-</th>
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<td>68</td>
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<td>10-24.9</td>
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<td>38</td>
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<td>.002</td>
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<td>89</td>
<td>106</td>
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<td></td>
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<tr>
<td>T</td>
<td>0</td>
<td>0</td>
<td>.88</td>
<td>55</td>
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Values are the number of cases in each group.
*Comparison of B-lineage cases only.
†Comparison of all cases.
#Non-L3, Tdt' ALL.
laboratory features of the patients according to the presence or absence of CD45 expression. Cases lacking the antigen had significantly lower leukocyte counts and serum LDH levels. Notably, blasts of CD45− patients were more likely to have leukemic cell hyperdiploidy with greater than 50 chromosomes or a DNA index greater than 1.15. Among ALL cases with hyperdiploidy greater than 50, the pattern was similar between the CD45' and CD45− cases (Table 2, data for CD45+ cases not given). The region of the CD45 antigen locus, 1q32, was duplicated in three of 25 CD45− cases.

CD45 antigen expression and age, sex, race, hepatomegaly, splenomegaly, hemoglobin, platelet count, leukemic blast morphology (FAB criteria), or presence of chromosomal translocation (data not shown).

Because all CD45− cases were B-lineage leukemia, similar comparisons with presenting features were made after excluding cases of T-ALL (Table 1). The absence of CD45 expression was still significantly associated with lower serum LDH levels, leukemic cell hyperdiploidy with greater than 50 chromosomes, and a DNA index greater than 1.15. In fact, an inverse linear correlation between CD45 expression and DNA index could be shown (Fig 2). At a median follow-up time of 2.7 years, patients with CD45− blasts tended to have a more favorable outcome than did those with CD45+ blasts (18/19 vs 86/111 patients remain event-free survivors, P = .09). Because of the wide range of CD45 levels, we determined whether the degree of antigen expression might correlate more closely with clinical features and outcome. Patients were divided into three groups as defined by CD45 positivity (group I, <20%; group II, 20% to 89%; group III, ≥90%). Leukemic cell hyperdiploidy with greater than 50 chromosomes (or DNA index >1.15) was found in 78% (22%), and 9% (46%).

No correlation by univariate analysis was found between CD45 antigen expression and age, sex, race. Univariate correlations between CD45 antigen expression and other clinical and laboratory features of the patients according to the presence or absence of CD45 expression. Cases lacking the antigen had significantly lower leukocyte counts and serum LDH levels. Notably, blasts of CD45− patients were more likely to have leukemic cell hyperdiploidy with greater than 50 chromosomes or a DNA index greater than 1.15. Among ALL cases with hyperdiploidy greater than 50, the pattern was similar between the CD45' and CD45− cases (Table 2, data for CD45+ cases not given). The region of the CD45 antigen locus, 1q32, was duplicated in three of 25 CD45− cases and six of 42 CD45+ cases with greater than 50 chromosomes (P = 1.0). No correlation by univariate analysis was found between CD45 antigen expression and age, sex, race, hepatomegaly, splenomegaly, hemoglobin, platelet count, leukemic blast morphology (FAB criteria), or presence of chromosomal translocation (data not shown).

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Fig 2. A smoothed, isotonic plot showing an inverse relationship between levels of CD45 expression and DNA index of cases of B-lineage ALL.

**DISCUSSION**

CD45 antigen expression was undetectable on lymphoblasts in 13% of newly diagnosed childhood ALL cases. Our results corroborate the observations of others showing that some cases of lymphoma or acute leukemia or leukemic cell lines may lack CD45.2"3 However, given the ubiquitous expression of this antigen on normal leukocytes and their precursors, the relatively high frequency of CD45 negativity in this study was unexpected. Although different isoforms of CD45 may be expressed on hematopoietic cells, the HLe-1 (clone 2D1) monoclonal antibody used in this study detects the framework epitope common to all members of the CD45 antigen family (CD45, CD45RA, CD45RB, CD45RO).3-29

Another intriguing finding was that all CD45- cases were of the B-cell lineage. Even among CD45+ cases, expression of the antigen was higher in T- than in B-lineage cases. During the course of normal B-cell development and differentiation, CD34 antigen expression progressively declines, while the expressions of CD45, CD20, and cytoplasmic immunoglobulins increase.24 In this study, there was an inverse correlation between CD34 and CD45 antigens, as expected. However, a disproportionately high percentage of CD45- cases (12/32) had cytoplasmic μ-immunoglobulin. Moreover, there was an inverse relationship between CD45 and CD20 antigen expression, contrary to findings in normal B-cell development.23,24 These results support the contention that most B-lineage leukemias exhibit "developmental asynchrony" as compared with their normal counterparts.5,30

Perhaps more interesting is the association between the lack of or low CD45 antigen expression and the presence of favorable presenting features of childhood ALL, including low leukocyte counts and serum LDH levels, leukemic cell hyperdiploidy with greater than 50 chromosomes, and a DNA index greater than 1.15.28 The low or absent CD45 expression was marginally associated with a good prognosis \( (P = .09) \). However, when cases were analyzed according to the degree of CD45 expression, a significant correlation with treatment outcome emerged. Similarly, in a limited study of childhood and adult ALL, Caldwell et al31 also suggested an inverse relationship between quantitative CD45 expression and therapeutic outcome. In their study, patients with the least intensely staining lymphoblasts appeared to have a more favorable response to therapy. That CD45 expression lost prognostic significance in the multivariate analysis is not surprising, since it is closely correlated with leukemic cell ploidy and DNA content, which are among the factors with the strongest prognostic impact.31

The biologic basis for CD45- blasts in a significant proportion of B-lineage ALL cases is not clear from our analyses. A possible explanation is that CD45- B-precursor cells do in fact express the antigen, but at surface densities too low for detection by flow cytometry.29 However, lymphoblasts from our CD45- cases did not have detectable CD45 antigen when studied by the sensitive alkaline phosphatase anti-alkaline phosphatase technique (unpublished data); studies to identify the mRNA for this glycoprotein are being performed. More difficult to reconcile is the association of hyperdiploidy and low expression of detectable CD45. One possibility is that the cellular cytoskeletal proteins that anchor the CD45 antigen39 are also important in orderly cell mitosis, so that defective skeletal proteins, as might be found in leukemic cells, would result in both hyperdiploidy and loss of CD45 in some cases of ALL.

In conclusion, we have shown that 13% of cases of newly diagnosed childhood ALL lack CD45 antigen expression and that these cases are of B lineage. We have further demonstrated a significant inverse relationship between leukemic blast CD45 antigen expression and blast cell ploidy and clinical outcome. Further studies are needed to define the functional basis for our observation that CD45- cases tend to be hyperdiploid.
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Lack of CD45 antigen on blast cells in childhood acute lymphoblastic leukemia is associated with chromosomal hyperdiploidy and other favorable prognostic features [see comments]

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