CD45 EXPRESSION AND PROGNOSIS IN ACUTE LYMPHOBLASTIC LEUKEMIA

To the Editor:

The recent publication by Behm et al concerning the relationship between prognosis and the percentage of CD45+ lymphoblasts in childhood acute lymphoblastic leukemia (ALL) was interesting, given our own interest in the CD45 antigen system. Since our earlier report suggesting the same inverse relationship between the quantity of surface CD45 on lymphoblasts and therapeutic outcome of children and adults, much has been learned. One of the more exciting findings is that the cytoplasmic tail common to all isoforms of CD45 possesses protein tyrosine phosphatase (PTPase) activity and works in a regulatory manner to modulate activity of certain members of the src family of tyrosine kinases, which are known to be involved in cellular transformation, thus suggesting a role for CD45 in malignancy.

At least 4, and possibly as many as 8, isoforms of CD45 that differ in relative molecular mass (M,) may be produced by alternative splicing of the messenger RNA in normal cells. Unfortunately, the sensitivity of gel electrophoresis and the small differences in M, of certain isoforms limits the ability to readily resolve these proteins. Therefore, emphasis has been placed on immunophenotypic analysis of cells using monoclonal antibodies (MoAbs) reactive with various isoforms of CD45 and with different epitopes on the same protein. MoAbs that react with a putative "backbone" structure common to all isoforms (as well as can be ascertained) are referred to as CD45, whereas those restricted protein segments (usually the N-terminal region) may be referred to as CD45RA, CD45RB, or CD45RO.

Behm et al used the CD45 MoAb HLe-I (clone 2D1; Becton Dickinson [BD], Mountain View, CA) and demonstrated that children with CD45- lymphoblasts (<20% positive cells) had a better prognosis than those with CD45+ lymphoblasts. By their definition, 13% of the cases were negative for CD45. We also used HLe-I in our earlier studies, but expressed our data as the fluorescence intensity (FI) or relative amount of MoAb binding to cells, rather than the percentage of positive cells. It is recognized that below a certain level of quantitative FI, cells will appear negative for a specific MoAb, and that this positive cut-off point can be altered by adjustments to the instrument, methodology, or different MoAb preparations.

We have continued to investigate CD45 expression in ALL using additional MoAbs reactive with the backbone structure, as well as certain isoforms. HLe-I (BD), J33 (AMAC, Westbrook, ME), KC56, and C-CD45 (Coulter Immunology, Hialeah, FL) are all classified as CD45. Based on the knowledge that CD45 FI correlates with stages of normal and malignant B-cell differentiation, it is expected that decreased levels will be found in cases of ALL. Using lymphoblasts from childhood ALL and a standardized direct immunostaining protocol, we found that cells from 44 of 52 cases bound HLe-I at greater than 20% positivity, similar to data reported by Behm et al, but that all cases expressed greater than 20% KC56, C-CD45, and J33 positivity (data not shown). Data from 21 representative cases of ALL, ordered in increasing CD45 FI, are shown in Fig 1 A and B. A discrepancy in percent CD45 positivity between MoAbs occurred when the linear FI of CD45 was very low (less than linear channel 5 after background subtraction), thus suggesting that the observation of Behm et al was indeed related to the sensitivity of their measurements rather than biologic CD45 negativity. In all cases but one, the FI of KC56, C-CD45, and J33 was greater than that of HLe-I. Further, when our...
cases with less than 20% HLe-1 positivity were amplified using a sandwich method of immunostaining, only 1 of 52 cases failed to become positive.

Given the known enzyme activity of CD45 and its interrelationship with src tyrosine kinases, the question arises as to whether the quantity of surface CD45 expressed relates to cell functions. It appears that decreased quantitative CD45 expression correlates with therapeutic outcome, whereas an increased level of the neutral endopeptidase CD10 correlates with outcome. In normal B cells, these two surface antigens are inversely related in quantitative terms, thus suggesting that the ALL blasts corresponding to the least mature stage of normal differentiation respond best to therapy. In general, this concept is supported by the observation that a hierarchy of responses exists that corresponds to stages of differentiation: B < pre-B < early B-precursor ALL. Overall, it is clear that standardized quantitation of cell surface antigens offers an added level of information and should be used whenever possible for immunophenotypic studies of normal and abnormal cells.

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REFERENCES

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RESPONSE

The comments of Caldwell et al are appreciated. Their study of CD45 in ALL adds another dimension to the understanding of this antigen's expression by leukemic lymphoblasts. In their experience, all of 52 cases of ALL were found to express CD45 when KC-56, C-CD45, and J33 (anti-CD45 common epitope MoAbs) were substituted for HLe-1. Their studies imply, and we agree, that reporting the percent positive blasts and using arbitrary cutoff points for positivity such as the 20% value used in our report do not accurately differentiate between very weak expressing and nonexpressing leukemic blast populations.

Since the submission of our report, we have analyzed an additional 118 cases of childhood ALL with HLe-1 (anti-CD45), Leu18 (anti-CD45RA), and PD7/26 (anti-CD45RB). Determination of percent positive blasts was performed by comparing the linear FI curves of the test and negative control antibodies. Similar to the results of our previous report, 16 of 118 (13.5%) cases had less than 20% of analyzed blasts reacting with HLe-1, Leu18, and PD7/26. Cytoplasmic μLg was strongly expressed in 8 of these 16 cases. In 12 of 16 cases, there were increases of the mean channel of linear fluorescent intensity for histogram curves of the CD45 test antibodies when compared with the curve of the negative control. These observations agree with those of Caldwell et al and support their impression that true CD45 antigen-expressing cases of ALL are very rare or may not exist. We further reexamined the blasts of 8 cryopreserved cases of ALL with very low or moderate levels of CD45 expression (5% to 40% positive blasts) with J33, KC-56, and HLe-1 MoAbs antibodies as used by Caldwell et al. Unlike their findings, we see no differences of CD45 antigen expression nor shifts in mean channels of the linear FI histograms among the three different MoAbs. Interestingly, all 8 cases appear to show slight increases of CD45 expression over that observed with noncryopreserved blasts tested at diagnosis.

Several findings in our report need to be emphasized. Firstly, very weak CD45 antigen expression in B-lineage ALL does not correlate with the stage of leukemic blast maturation. No difference in the number of cases with very weak blast CD45 antigen expression is observed between early pre-B (μLg<sup>-</sup>) and pre-B (μLg<sup>+</sup>) cases of ALL. Secondly, CD45 antigen status does not correlate with patient clinical outcome when multivariant analysis corrects for blast cell DNA index. Lastly, the most interesting finding in our investigations is the significant association of low CD45 antigen expression with increased DNA index (DNA index >1.15) and chromosomal hyperdiploidy. We feel that further investigations of CD45 antigen expression of B-lineage ALL should be directed towards defining this latter association and looking to see if functional differences exist between leukemic lymphoblasts with low and high CD45 antigen densities.

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CD45 expression and prognosis in acute lymphoblastic leukemia [letter; comment]

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