CD58 Expression Decreases as Nonmalignant B Cells Mature in Bone Marrow and Is Frequently Overexpressed in Adult and Pediatric Precursor B-Cell Acute Lymphoblastic Leukemia

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Abstract

We used flow cytometry to determine the CD58 expression on nonmalignant B cells at different stages of maturation in the bone marrow and compared it with that of blasts in adult and pediatric precursor B-cell acute lymphoblastic leukemia (B-ALL). The mean fluorescence intensity (MFI) of CD58 expression decreased significantly as nonmalignant B cells differentiated in the bone marrow from an early to a mature stage. Few nonneoplastic B cells at a mid or mature stage of development expressed CD58 MFI values comparable to those seen in leukemic cases. Early-stage nonneoplastic B-cell precursors expressed relatively higher CD58 levels, which frequently overlapped with the variable level of CD58 expression observed among leukemic blasts. As a group, however, the malignant precursor B-ALL cells showed significantly higher expression of CD58 than nonmalignant B-cell populations at any maturational stage. These findings support the potential usefulness of CD58 expression in the diagnosis and monitoring of precursor B-ALL, but only when blasts express high levels of CD58.

CD58 (lymphocyte function–associated antigen 3) is a cell surface adhesion molecule that binds to CD2 on T cells, an interaction that results in several immunomodulatory effects.1-3 CD58 is distributed widely among hematopoietic and non-hematopoietic cells and in a variety of malignant neoplasms, including chronic B-cell leukemias,4 neoplastic T cells,5 Reed-Sternberg cells,6 myeloma,7 and myeloid leukemias.8 CD58 has been reported to be overexpressed in cases of precursor B-cell acute lymphoblastic leukemia (B-ALL). De Waele and colleagues9 compared the expression of several adhesion molecules, including CD58, in CD34-expressing acute myeloid and lymphoblastic leukemia cells with benign CD34+ myeloid and lymphoid counterparts. These authors demonstrated that the expression of CD58 by precursor B-ALL was greater than the CD58 expression by CD34+ benign B-cell precursors.

Chen et al10 identified and described CD58 overexpression in cases of pediatric precursor B-ALL by complementary DNA array analysis. Their results demonstrated the potential application of array-based technology for discovering useful markers in the diagnosis of disease and the possibility of using CD58 protein expression for minimal residual disease (MRD) monitoring in precursor B-ALL. The possible advantage of using the quantitative CD58 expression level in the detection of MRD in B-ALL recently was confirmed by Veltroni et al.11

Previous studies have addressed the immunophenotypic properties of benign precursor B cells and precursor B-ALL using multiparameter flow cytometry.9,10,12-14 McKenna et al13 analyzed 662 bone marrow samples and described the phenotype of precursor B cells (hematogones) using CD34, CD10, CD19, and CD20 antibodies. With these reagents, nonneoplastic B-cell precursors can be divided into distinct maturational stages, including early, mid, and mature stages.13,14
The purposes of our study were to use multiparameter flow cytometry to characterize the expression of CD58 on nonmalignant precursor B cells as they traverse the various maturational stages in the bone marrow, determine the expression of CD58 on malignant precursor B-ALL samples from adult and pediatric patients and compare it with the expression of CD58 on nonmalignant precursor B cells, and assess the expression of CD58 on malignant precursor B-ALL from different sites (bone marrow, peripheral blood, and cerebrospinal fluid [CSF]).

Materials and Methods

Sample Selection

All clinical samples submitted for flow cytometric analysis to the Hematopathology Laboratory, Shands Hospital, University of Florida, Gainesville, from December 2001 through May 2003 were screened for the presence of characteristic nonmalignant B cells as described in the following text. All cases of precursor B-ALL from July 2001 to May 2003 were used for comparison. A total of 140 specimens (bone marrow, 129; peripheral blood, 10; CSF, 1) were studied with approval by the University of Florida Institutional Review Board.

Nonmalignant samples included 104 bone marrow samples that contained variable but distinct, normal, maturing B-cell populations, as judged by the increasing expression of CD58 on malignant precursor B-ALL from different sites in the bone marrow, determine the expression of CD10+/CD19+ precursor B-ALL. Fifteen samples were newly diagnosed cases (8 bone marrow and 7 peripheral blood samples), and 21 were recurrent or residual disease cases (17 bone marrow, 3 peripheral blood, and 1 spinal fluid sample).

Sample Processing and Analysis

Bone marrow aspirate and peripheral blood samples first were exposed to erythrocyte lysing buffer (8.29 g of NH₄Cl, 1.0 g of KHCO₃, 37 mg of EDTA, 1 L distilled water) for 10 minutes at room temperature at a ratio of 1 mL of sample/9 mL of lysing buffer. The samples were centrifuged at 500 g for 5 minutes, the supernatant was aspirated, and the cells were resuspended in phosphate-buffered saline with 0.1% NaN₃ (PBS) and washed twice in PBS. Cells (3 × 10⁷) were added to albumin-precoated wells (Sigma Chemical, St Louis, MO) in Falcon 96-well U-bottom assay plates (BD Labware, Franklin Lakes, NJ) with 20 µL of appropriate dilutions of multiple fluorochrome-conjugated antibodies in Hanks balanced salt solution (Mediatech Cellgro, Herndon, VA) with 50% human AB serum (Bio-Whittaker, Walkersville, MD).

The antibodies used are listed in Table 2. The trays were incubated for 15 minutes in the dark on ice. Subsequently, 50 µL of PBS was added to each well, and the trays were centrifuged at 500g for 5 minutes. The supernatants were discarded, 100 µL of PBS was added, and the trays were centrifuged again at 500g for 5 minutes. This cell-washing procedure was repeated twice.

After the last centrifugation and supernatant were discarded, cells were transferred to microtubes in a final volume of 250 µL of PBS or To-Pro-3 iodide (Molecular Probes, Eugene, OR). The latter was used for viability gating as needed. The microtubes were inserted into corresponding 12 × 75-mm tubes in the loader rack of a flow cytometer (FACScalibur, Becton Dickinson [BD], San Jose, CA), and 30,000 events per
tube were collected. The data were acquired using CellQuest software (BD). Daily calibration of the instrument was performed using standardized CaliBRITE Beads (BD) with FACSComp Software (BD), and compensation was performed using appropriately stained normal peripheral blood samples.

Data Analysis

The analysis was performed using WinMDI software (Scripps Research Institute, San Diego, CA; http://facs.scripps.edu/software.html). Initial cell selection (gating) was established using CD45 expression and side-scatter (SSC) properties to exclude granulocytes, nucleated RBCs, and monocytes from the analysis of precursor and mature lymphocytes. Nonmalignant B cells were further identified and categorized according to maturational stages. Nonmalignant B cells were designated as early (CD45dim/CD34+/CD10+/CD19+/CD20–), mid (CD45intermediate/CD34–/CD10+/CD19+/CD20–/+), and mature (CD45bright/CD34–/CD10–/CD19+/CD20+). The CD58 expressions of the various populations are shown in Figure 1. The CD58 MFI of early B-cell precursors was 49.8 ± 17, which was significantly greater than that of midstage B cells (26.4 ± 10.1) (P < .0001). CD58 MFI expression of the latter was significantly greater than that of mature B cells (12.6 ± 6.4) (P < .0001).

CD58 Expression by Nonmalignant B Cells

The expression of CD58 by precursor B-ALL in bone marrow was significantly higher than that of nonmalignant B

Statistical Analysis

Descriptive summary statistics included the mean, SD, and range of values. The Mann-Whitney U test was used to compare the CD58 expression of the different cell populations (GraphPad Software, San Diego, CA). Unless stated otherwise, results are given as mean ± SD.

Results

CD58 Expression by Nonmalignant B Cells

Image 1 illustrates nonmalignant B-cell maturational stages in a typical fresh bone marrow specimen. Nonmalignant B cells were designated as early (CD45dim/CD34+/CD10+/CD19+/CD20–), mid (CD45intermediate/CD34–/CD10+/CD19+/CD20–/+), and mature (CD45bright/CD34–/CD10–/CD19+/CD20+). The CD58 expressions of the various populations are shown in Figure 1. The CD58 MFI of early B-cell precursors was 49.8 ± 17, which was significantly greater than that of midstage B cells (26.4 ± 10.1) (P < .0001). CD58 MFI expression of the latter was significantly greater than that of mature B cells (12.6 ± 6.4) (P < .0001).

CD58 Expression in Precursor B-ALL

The expression of CD58 by precursor B-ALL in bone marrow was significantly higher than that of nonmalignant B
CD58 expression of nonneoplastic B cells in early, mid, and mature stages of development and in precursor B acute lymphoblastic leukemia (ALL). The bars mark the mean and SD. Among the nonneoplastic B cells, CD58 expression was greatest for the least mature cells and decreased as B cells matured. Precursor B-ALL blasts expressed higher values than any nonneoplastic B cells. Differences in mean fluorescence intensity (MFI) among the nonneoplastic B-cell groups and between each of the nonneoplastic B cells and the precursor B-ALL were statistically significant (P < .0001 for all groups).

cells (P < .0001) for all stages of B-cell maturation (Figure 1). However, there was a considerable overlap in the CD58 MFI between early-stage nonneoplastic precursor B cells and leukemic blasts. CD58 MFI expression in early nonneoplastic B cells ranged from 20.4 to 126.2, whereas the expression in leukemic cases in bone marrow ranged from 41.3 to 329.6. Approximately one half (12/25 [48%]) of the leukemic cases exceeded the upper limit of CD58 expression by nonneoplastic B cell precursors. Although we did not measure each individual plot, we did not recognize obvious qualitative differences in CD58 expression patterns between malignant and individual stages of benign B-cell precursors.

Leukemic blasts coexpressed CD34 in most cases (22/25 [88%] of marrow samples, 8/10 [80%] of the peripheral blood samples, and in the single CSF sample). There were no statistical differences in CD58 MFI expression between CD34+ (131.7 ± 67.4) and CD34− (113.5 ± 71.5) blasts (P = .49). In 1 of 3 CD34− cases in the bone marrow, the blasts expressed CD58 at a level below the upper limit of expression observed among midstage nonneoplastic precursor B cells. The CD58 MFI expression for all samples of precursor B-ALL cells, including bone marrow, peripheral blood, and CSF, was 129.2 (± 67.2). The expression of CD58 in circulating precursor B-ALL blasts was lower (99.2 ± 49.5) than that of blasts in bone marrow samples (144.7 ± 68.8), but the difference was not statistically significant (P = .068) (Figure 2).

The CD58 expression in blasts from the single CSF (42.7) was lower than that in most blasts from bone marrow or peripheral blood. We observed no significant differences in CD58 expression between the blasts of adult (132.5 ± 63.2) and pediatric (127.9 ± 69.9) samples (P = .61), and there were
no statistically significant differences between CD58 expression of precursor B-ALL blasts at diagnosis and at recurrence (data not shown).

Discussion

Previous work by De Waele and colleagues\(^9\) compared the expression of CD58 and several other adhesion molecules in bone marrow samples of CD34+ acute myeloid and lymphoblastic leukemia cells with nonneoplastic CD34+ myeloid and lymphoid counterparts. These authors demonstrated that the expression of CD58 by precursor B-ALL was greater than the CD58 expression by normal CD34+ B-cell precursors and further suggested that abnormal adhesive capacity of leukemic cells might influence proliferation, localization, and apoptotic properties of acute myeloid and acute lymphoblastic leukemia. By using flow cytometry, Chen et al\(^10\) demonstrated that the expression of CD58 protein was abnormally high in a substantial proportion of B-lineage ALL and that CD58 expression in combination with CD19, CD34, and CD10 was useful to identify residual leukemic cells in bone marrow samples from patients in clinical remission. Our findings are in agreement with these reports.

More recently, Veltroni et al\(^11\) studied a large series of bone marrow samples with ALL at diagnosis, early-B stage, mature-B stage, and during follow-up and compared results with those obtained from normal and regenerating marrow samples. No significant differences between regenerating and normal B lymphocytes were observed, but CD58 expression was significantly higher in ALL blasts than in normal B lymphocytes in the marrow. The authors indicated that compared with normal B cells, CD58 was overexpressed in 93.5% of the precursor B-ALL cases using fluorospheres to calculate molecules of equivalent soluble fluorochrome and recommended its use in MRD detection of precursor B-ALL. The results confirmed the usefulness of CD58 overexpression as a marker for MRD studies and also showed that a large number of precursor B-ALL cases have levels of CD58 expression similar to those in normal cells. However, because the authors indicated that the analysis was carried out on all CD19-expressing cells, it should be assumed that the measurements encompassed all stages of normal B-cell development, including mature B cells.

Therefore, we studied the expression of CD58 in nonmalignant B cells at different stages of differentiation in the bone marrow and compared the results with those of leukemic precursor B-ALL blasts. We wanted to determine whether CD58 was truly overexpressed in precursor B-ALL or merely a reflection of a maturational stage. We found that CD58 expression was highest on the early nonneoplastic B-cell precursors and decreased as B cells matured in the bone marrow, suggesting that CD58 expression is a marker of B-cell immaturity.

Confirming earlier results by other investigators\(^9-11\) and extending the observations to include adult samples, we showed that as a group, precursor B-ALL blasts expressed significantly higher average CD58 levels than nonmalignant B cells of all maturational stages. However, approximately half of the leukemic cases had a CD58 expression level similar to that of the earliest stage of nonneoplastic B cells. These observations support the notion that CD58 overexpression could be added to currently popular antibody combinations for detecting MRD in precursor B-ALL and might be useful in approximately half of the cases (those that overexpress CD58).

Because the measurements of antibody binding by flow cytometry have not been standardized and different methods are used to calculate antigen expression, individual laboratories would have to develop their own MFI cutoff values if CD58 were to be used in differentiating precursor B-ALL and normal B-cell precursors using a quantitative approach such as the one described herein. However, because CD58 most likely will be informative mainly when previously shown to be overexpressed on the B-ALL blasts, a qualitative comparison between the diagnostic and follow-up samples also might...
be adequate, provided that CD58 expression is found to be stable over time on paired diagnostic and relapse samples from the same patient (a potential subject of future studies). Although the number of cases was relatively low, we observed no differences in CD58 expression between CD34+ and CD34– ALL blasts. Thus, in cases of CD34– leukemia, there may be less difficulty in differentiating neoplastic cells from normal cell counterparts in the marrow because nonneoplastic B cells at the mid or late stage of development show a relatively low level of CD58 expression.

The biologic significance of CD58 overexpression in leukemic cells is not clear. CD58 is an adhesion molecule that is expressed at different levels in a variety of normal and neoplastic cells and might have a role in their interaction with regulatory immune cells and tissue localization. As with other cell adhesion molecules, CD58 might participate in cell-cell and cell-matrix adherence. An abnormal expression of adhesion molecules on leukemic cells might alter their contact with stromal cells and have a role in their ability to migrate out of the bone marrow. Earlier studies demonstrated that in ALL, the expression of CD58 was correlated inversely with the percentage of peripheral blast cells. In this regard, it is of interest that although the differences were not statistically significant, most likely owing to the relatively few samples studied, in our study CD58 expression tended to be lower in peripheral blood samples (and in 1 CSF sample) compared with bone marrow samples. Therefore, it might be of interest in a future study to correlate the level of CD58 expression with the peripheral blast count.

We have shown that CD58 expression decreases as nonneoplastic B cells mature in the bone marrow and independently confirmed that when CD58 is highly overexpressed, it may be used to differentiate normal precursor B cells from precursor B-ALL blasts.

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References


