Expression and Structure of CD22 in Acute Leukemia

By Daniel R. Boué and Tucker W. LeBien

The purpose of this study was to examine the expression and structure of CD22 in B cell precursor acute lymphoblastic leukemia (BCP-ALL), acute myeloid leukemia (AML), and T cell acute lymphoblastic leukemia (T-ALL). By using immunofluorescence microscopy and flow cytometry we observed that CD22 is expressed not only in the cytoplasm (as previously reported) but also on the cell surface of virtually all (15/16) BCP-ALL examined. CD22 that was biosynthetically labeled with 35S-cysteine and immunoprecipitated from the uncommon cytoplasmic CD22-positive/surface CD22-negative BCP-ALL cells was analyzed by single-dimension sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Our results indicated that the cytoplasmic form of CD22 comigrated with [125I]-lactoperoxidase-labeled surface CD22. Therefore, cytoplasmic CD22 is probably a pool of fully processed glycoprotein. We also observed unusual cases of AML (~20%) that expressed cytoplasmic CD22 based on immunofluorescent staining; however, biosynthetic labeling and immunoprecipitation revealed an apparently cross-reactive protein(s) of ~250 to 300 kd in AML cells. No T-ALL cell lines examined expressed either cytoplasmic or surface CD22. Thus, cytoplasmic and surface expression of bone fide CD22 appears restricted to B cells, which suggests that this molecule subserves a function unique to B cells.

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MATERIALS AND METHODS

Cells and monoclonal antibodies. Newly diagnosed, fresh BCP-ALL (CD19-positive/HLA-DR-positive) bone marrow specimens consisting of >90% leukemic blasts (as determined by Ficoll-hypaque interface cell >90% reactive with either anti-CD19 and/or anti-CD10 antibodies) were obtained from the Cell Marker Laboratory, Department of Laboratory Medicine and Pathology, University of Minnesota. AML bone marrow specimens consisting >90% leukemic blasts were obtained fresh or cryopreserved through the aforementioned laboratory. Bone marrow specimens were obtained after review by the committee on human subject research. Diagnosis was based on standard morphological, cytochemical, and immunologic criteria.

Established leukemic cell lines were maintained in RPMI 1640 containing 5% or 10% fetal bovine serum. B-ALL cell lines examined included KOPN-1, HPB-Null, Reh, NALM-6, NALM-16, KM-3, LAZ-221, and BLN-1 (B. Wörmann et al, manuscript submitted). T cell acute lymphoblastic leukemia (T-ALL) cell lines examined included HSB-2, MOLT-4, HPB-MLT, HPB-ALL, RPMI-8402, and CEM. Myeloid leukemic cell lines examined included K562, HL-60, KG-1, and ML-2.10 The Raji-Burkitt's lymphoma cell line10 and the RS4;11 biphenotypic cell line11 were also used.

Purified Leu 14/anti-CD22 was the generous gift of Dr Lewis Lanier and Becton Dickinson Immunocytometry Systems, Mountain View, CA. ToI5/anti-CD22 was purchased from Dakopatts, Copenhagen. HD39/anti-CD22 was obtained through the Third International Workshop on Human Leucocyte Differentiation Antigens (London, 1986). B1-biotin/anti-CD20 was purchased from Coulter Immunology, Hialeah, FL. W6/32/anti-class I17 was produced from hybridoma cells obtained through the American Type Culture Collection, Rockville, MD, and purified from ascitic fluid on protein A-Sepharose CL-4B (Pharmacia Fine Chemicals, Piscataway, NJ) according to the method of Eyt et al.18 Purified isotype-identical control myeloma proteins of the IgG2a (UPC 10) and IgG2b (OPC 195) subclasses were obtained from Litton Bionetics, Charleston, SC. Other antibodies used included BA-3/anti-CD10,13 TA-1/anti-CD11a,1a and BA-5/anti-CD21.6 Fluorochrome-labeled antibodies included fluorescein isothiocyanate–conjugated goat anti-mouse Ig (FITC-GAM; Cappel Worthington Biochemicals, Mal-
with a slides were read or 2 each) of Leul4, Tol5, or CD22. Patients with <

cule (see below) we found that almost all BCP-ALL (CD 19-

have collectively indicated that newly diagnosed

5 were fixed with acetone for 1 previously described.6

were also labeled by 251-lactoperoxidase-catalyzed iodination as

slides were rinsed twice with PBS and immediately stained

biochemically analyzing the cytoplasmic form of the mole-

process of examining BCP-ALL for CD22 expression, before

and BCP-ALL cell lines are predominantly cyto-

grams set for each.

Fig 1. Dual-color fluorescence histograms of five representa-
tive BCP-ALLs simultaneously analyzed for anti-CD22/FITC (green)

and anti-CD20/PE (red) fluorescence. Light scatter profiles indi-
cated that a single bulk population of lymphoblastic cells was

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plasmic CD22-positive/surface CD22-negative.1,4,5,8 In the process of examining BCP-ALL for CD22 expression, before

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Results

Expression and structure of CD22 in BCP-ALL. Several reports have collectively indicated that newly diagnosed BCP-ALL and BCP-ALL cell lines are predominately cytoplasmic CD22-positive/surface CD22-negative.1,4,5,8 In the process of examining BCP-ALL for CD22 expression, before biochemically analyzing the cytoplasmic form of the molecule (see below) we found that almost all BCP-ALL (CD19-positive/HLA-DR-positive) examined expressed surface CD22. Patients with >50% positive cells were considered positive. There were no patients between 10% and 50% positive. Fifteen of 16 samples were surface CD22-positive when stained with Leu14/anti-CD22 and analyzed by immunofluorescence microscopy, and all 16 were cytoplasmic CD22-positive. We also noted that surface CD22 staining was brighter on CD20-positive (4/16) than on CD20-negative samples. To examine this more quantitatively, five representative cases were analyzed by dual-color flow cytometry for CD22 and CD20. The results in Fig 1 show that patients 1 to 5 were all clearly surface CD22-positive; the
mean channel numbers of fluorescence intensity ranged from 296 to 365. The highest level of CD22 expression was on the single CD20-positive patient (no. 1) examined by flow cytometry. Patient 5 was the only CD10-negative BCP-ALL among the 16 patients examined (data not shown). We also found that eight of nine BCP-ALL cell lines were surface CD22-positive, seven of the eight positive cell lines being >90% surface CD22-positive. The single surface CD22-negative/cytoplasmic CD22-positive cell line was KM-3.

The results in Fig 1 were obtained by using saturating concentrations of Leu14/anti-CD22. Because of the inconsistency of these findings with respect to previous reports,\textsuperscript{4,5,8} we considered the possibility that different anti-CD22 antibodies may either (a) react with epitopes exhibiting variable surface expression on BCP-ALL or (b) have significantly different binding affinities. The latter might lead to contradictory findings if the antibodies were not used at saturating concentrations. We therefore compared To15/anti-CD22, previously reported to have minimal reactivity with newly diagnosed BCP-ALL and BCP-ALL cell lines,\textsuperscript{1,5} with the Leu14/anti-CD22 used above, which reacts with a different epitope on CD22.\textsuperscript{17} Identical concentrations of Leu14 and To15 were tested on three different target cells (NALM-6, Raji, and HSB-2), and the results are shown in Fig 2. Both antibodies showed similar staining patterns on the three cell lines when used at saturating concentrations (5.0 µg/10⁶ cells); however, Leu14 appears to have a higher affinity for CD22 because with identical suboptimal antibody concentrations (0.1 µg/10⁶ cells), Leu14 stained 85% of NALM-6 cells while To15 weakly stained 34% of NALM-6 cells. Similar results were seen with RAJi cells (Fig 2). Saturating levels of Leu14, To15, and HD39—previously reported to react with surface CD22 in only rare (<10%) cases of BCP-ALL and BCP-ALL cell lines—reacted identically with all nine BCP-ALL cell lines tested above (8/9 positive; data not shown).

The structure of cytoplasmic CD22 was examined by \textsuperscript{35}S-cysteine biosynthetic labeling of surface CD22-negative Reh cells isolated by cell sorting and the cytoplasmic and surface CD22-positive Raji cell line. The dense, perinuclear cytoplasmic CD22 staining in sorted surface CD22-negative Reh cells is shown in Fig 3A. Leu14 and isotype-identical control immunoprecipitates were examined by SDS-PAGE. As shown in Fig 4, Leu14/anti-CD22 immunoprecipitates (120/130-kd doublets) obtained after two- or five-hour pulse labeling of Reh and Raji cells are indistinguishable, at least
Fig 3. Photomicrographs of sorted surface CD22-negative Reh cell cytocentrifuge preparations stained with Leu14- or IgG2b-negative controls. Photomicrographs were taken with a Zeiss 35-mm camera using 160 ASA Tungsten Kodak color film (DIN, 23; magnification × 252.
by single-dimension SDS-PAGE. The longer pulse time required to label cytoplasmic CD22 in Reh cells is likely due to the slower rate of protein synthesis in these cells because incorporation of trichloroacetic acid (TCA) precipitable counts of $^{35}$S-cysteine/10$^6$ Reh cells was only 20% to 25% of that achieved by the same number of Raji cells for each pulse time shown. Five-hour, mock-pulsed, surface CD22-negative Reh cells were <2% surface CD22-positive when re-stained with Leu14/FITC-GAM and analyzed by flow cytometry. Leu14 immunoprecipitates from surface $^{125}$I-lactoperoxidase–labeled Raji, $^{35}$S-cysteine–labeled Raji, and $^{3}$H-cysteine–labeled sorted, surface CD22-negative Reh cells were also indistinguishable, at least by single-dimension SDS-PAGE (Fig 5), which supports our conclusion that cytoplasmic CD22 is probably identical to surface CD22 and likely represents a fully processed pool of CD22 glycoprotein.

Expression and structure of CD22 in AML and T-ALL. Mason et al reported that a small percentage of AML/AMML react with anti-CD22 by immunoenzymatic staining. We examined 13 newly diagnosed AML (one M1, six M2, one M3, and five M4 by French-American-British classification) and four AML cell lines for reactivity with anti-CD22. Three newly diagnosed AML (one M2, one M3, and one M4) exhibited moderate to intense cytoplasmic reactivity in >90% of all cells, and one AML cell line (ML2) was weakly reactive when using Leu14 and/or To15. In contrast, IgG2b myeloma protein and BA-5/anti-CD21 were completely unreactive. None of the AML exhibited any surface staining with anti-CD22. The intensity of cytoplasmic staining in fresh AML cells was similar to the intensity of cytoplasmic (CD22) staining in BCP-ALL cells; however, the cytoplasmic reactivity of anti-CD22 antibodies with AML cells was more dispersed than with BCP-ALL cells, which appeared to have a single, mostly perinuclear pool of glycoprotein (Fig 3A). All six T-ALL cell lines examined were both cytoplasmic and surface CD22-negative.

To ascertain whether cytoplasmic reactivity with anti-CD22 in AML represented bona fide CD22 expression, $^{35}$S-cysteine biosynthetic labeling of the most brightly "positive" newly diagnosed AML and the ML2 cell line was carried out. As shown in Fig 6, the Leu14 immunoprecipitate from the newly diagnosed AML revealed a higher–mol wt glycoprotein(s) of ~250 to 300 kd compared with the 120/130-kd doublet observed in surface CD22–negative Reh cells and the surface CD22–negative KM-3 cell line. The weak bands in the KM-3/CD22 lane between 130 and 200 kd were also present in the KM-3/2b control lane when the dried gel was exposed for a longer period of time. The Leu14 immunoprecipitate from the ML-2 cells also revealed a high–mol wt glycoprotein(s) of ~250 kd (data not shown). TCA-precipitable counts of $^{35}$S-cysteine/10$^6$ cells for the AML and ML-2 cell lysates were roughly equivalent to those for the Reh and KM-3 lysates. The high–mol wt glycoprotein(s) precipitated with Leu14 from the AML and ML-2 cells were not observed in class 1 immunoprecipitates from the same cells, thereby

Fig 5. Reducing SDS-PAGE (7.5%) comparison of Leu 14/anti-CD22 immunoprecipitates from $^{125}$I-lactoperoxidase–labeled Raji lysate and $^{35}$S-cysteine biosynthetically labeled Raji and sorted surface CD22–negative Reh lysate. The results are from a single experiment run on a single gel. The precipitate formed with equal microgram quantities of isotype-identical IgG2b (negative control) myeloma protein is shown in lanes labeled 2b. The relative mobility of Coomassie-stained protein standards is indicated on the y-axis.

Fig 6. Reduced SDS-PAGE (7.5%) fluorographic comparison of Leu 14/anti-CD22 immunoprecipitates from sorted surface CD22 negative Reh, unsorted surface CD22 negative KM3, and cells from an anti-CD22 reactive AML pulsed for 8 hours with $^{35}$S-cysteine. The results are from a single experiment run on a single gel. The precipitate formed with equal μg quantities of isotype identical IgG2b (negative control) myeloma protein is shown in lanes 2b. The relative mobility of Coomassie stained protein standards is indicated on the y-axis.
confirming their specific association with anti-CD22 (data not shown).

**DISCUSSION**

We have drawn three major conclusions from the data in this report. First, we found that CD22 is expressed on the surface of virtually all BCP-ALL examined in contrast to previous reports.8,9,12 Fifteen of 16 patients were clearly surface CD22-positive (Fig 1), and these results were apparently not attributable to differential epitope expression (Fig 2). Surface CD22 expression in BCP-ALL is therefore in marked contrast to what has been observed in putative normal bone marrow B cell precursor counterparts.9,12 The reason for this difference is not obvious, but it is possible that the predominant leukemic clone in BCP-ALL derives from a minor subpopulation of surface CD22-positive normal B cell precursors.

Second, we found that cytoplasmic CD22 probably represents a pool of fully processed glycoprotein, identical to surface CD22, at least by single-dimension SDS-PAGE (Figs 4 and 5); structural identity has also been reported for cytoplasmic and surface CD3.13 Cytoplasmic-to-surface transport of CD22 may represent a differentiation event in normal B cell precursors. Our previous examination of CD22 suggested that, although the rate of CD22 protein synthesis was normal, there might be an unusually slow rate of processing of this molecule compared with other lymphocyte membrane proteins.8 A slow rate of processing might result in a slow rate of membrane insertion, thereby facilitating detection of CD22 in the cytoplasm. This hypothesis might include the expression of very low levels of surface CD22 in normal B cell precursors that slowly accumulate to detectable levels as B cells differentiate. A slow rate of membrane insertion is consistent with the observation of others that both normal and malignant cells that become surface CD22-positive remain detectably cytoplasmic CD22-positive.14 Our findings with newly diagnosed BCP-ALL and BCP-ALL cell lines examined in this study are concordant with those results. Similarly, surface CD3-positive T-ALL remain detectably cytoplasmic CD3-positive.15

Cytoplasmic CD22 may also represent a reservoir of glycoprotein, and cytoplasmic-to-surface transport of CD22 may occur in response to an activation signal. Mature normal B cells activated with anti-Ig or protein A and malignant B cells activated with TPA exhibited increased surface CD22 expression. Surface CD22 in these situations may be derived from a preformed cytoplasmic reservoir, similar to how increases in surface CR1 and CR3 occur subsequent to neutrophil activation.20,21 Because one report has implicated a role for CD22 in B cell activation,22 this idea should be further pursued. Another explanation for cytoplasmic expression of CD22 is that it might represent surface CD22 that has been internalized in response to an unknown stimulus, analogous to internalization of class I antigens on T cells.23 Thus, cytoplasmic CD22 might be a pool of recycling glycoprotein, with surface expression increased by some stimuli and decreased by other as yet unidentified stimuli.

An alternative explanation for cytoplasmic expression is that CD22 might need to noncovalently associate with another molecule to facilitate or permit membrane insertion. Such an interaction occurs between β2-microglobulin and class 1 heavy chain24 and between CD3 and α/β T cell receptor components25; excess β2-microglobulin and CD3 are produced in these situations. Excess CD22 might remain in the cytoplasm after surface expression, an alternative explanation for why mature B cells express CD22 both in the cytoplasm and on the surface.14

The third conclusion we drew is that expression of structurally bona fide cytoplasmic CD22 is restricted to B cells because T-ALL cell lines do not express cytoplasmic CD22 and anti-CD22-positive AML cells express a cross-reactive, high-mol wt glycoprotein(s) (Fig 6). This cross-reactive glycoprotein may be more abundant or conformationally accessible in certain AMLs. Because antibodies to different CD22 epitopes reacted with the AMLs, it is possible that some degree of homology exists between CD22 and this high-mol wt glycoprotein. Serological cross-reactions between lymphocyte cell surface and cytoplasmic proteins have been previously described.26-27 The structural or functional significance, if any, of the CD22–cross-reactive cytoplasmic glycoprotein in AML remains to be determined.

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