Anti-CALLA Antibodies Identify Unique Antigens on Lymphoid Cells and Granulocytes

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The common acute lymphoblastic leukemia antigen (CALLA) is a 100-kd surface glycoprotein that is present on normal and malignant lymphoid cells. It is a useful marker for distinguishing between clinically important types of acute leukemia. Anti-CALLA monoclonal antibodies (MoAb) also react with mature myeloid cells (granulocytes), where they identify an antigen having a similar molecular weight (mol wt). We now report that the antigens detected by anti-CALLA MoAb on human lymphoid and myeloid cells differ in their behavior and chemistry. Surface-labeling studies indicate that the antigen on lymphoid cells has a mol wt of approximately 100 kd vs 110 kd for that on granulocytes. When cells are metabolically labeled with 35S-methionine, differences in the mol wt of these antigens are again observed. Unlike the lymphoid antigen, expression of that on purified granulocytes is not modulated by incubation with specific antibody. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of proteolytic digests of the two antigens fails to clarify their chemical relationship. Thus the antigens detected on these two cell types may share an epitope(s) but be chemically distinct, or CALLA may exist in distinct forms and behave differently on lymphoid cells and granulocytes.

MATERIALS AND METHODS

Antigens. Five CALLA-specific MoAb were used (anti-CALLA-1 (J5), anti-CALLA-4, anti-CALLA-5, anti-CALLA-6, and anti-CALLA-7). Hybridomas producing these MoAb were raised by immunization of BALB/c mice with CALLA-positive malignant cells. Anti-CALLA-1 was raised to leukemic cells from a patient with ALL, anti-CALLA-4/-5 and -6 to the ALL cell line, and anti-CALLA-7 to the Burkitt cell line. All identify a similar 100-kd surface glycoprotein and show identical reactivity patterns with cells from a panel of 38 acute leukemias and 32 hematopoietic cell lines. Modulation of CALLA by our initial anti-CALLA MoAb (J5) inhibits binding of the four other CALLA-specific MoAb. MoAb were obtained from either cell culture supernatant or ascites from tumor-bearing mice. They were ultracentrifuged and filtered before use.

Cells. Normal granulocytes were isolated by density gradient centrifugation on Ficoll-Diatrizoate (LSM, Litton Bionetics, Kensington, Md). Wright-Giemsa staining confirmed that cell purity was greater than 90%. Viability was greater than 95%, as measured by trypan blue exclusion. L221 and NALM-6 are CALLA-positive ALL cell lines.

Immunochemistry analysis. Binding of MoAb to target cells populations at 4°C was determined using a standard indirect immunofluorescence assay and monitored on the fluorescence-activated cell sorter (FACS IV, Becton Dickinson, Mountain View, Calif), with fluorescence displayed in the logarithmic mode. Before analysis, cells were suspended in RPMI 1640 containing 5% human AB serum to block Fc receptor sites. MoAb were used at a 1:100 dilution of ascites fluid to ensure antibody excess.

Antigenic modulation. Modulation of CALLA by specific MoAb was performed as described previously. Filter-sterilized anti-CALLA or isotype-matched control MoAb (100 μg/mL per 1 x 106 cells) was added to appropriate cultures, followed by incubation at 37°C for 16 hours. The extent of modulation was monitored by indirect immunofluorescence using the FACS.

Identification of antigens. Cell surface radiolabeling with 125I and immune precipitation of detergent-solubilized cell extracts were performed using standard methods. Antigens were resolved by electrophoresis on a 7% to 20% gradient sodium dodecyl sulfate-polyacrylamide slab gel (SDS-PAGE) and visualized by autoradiography. A ratio of 1 mCi of 125I to 50 x 106 cells was used. For metabolic labeling, freshly purified granulocytes or NALM-6 cells were incubated with 1 mCi of 35S-methionine per 50 x 106 cells for 16 hours at 37°C in methionine-free media. Detergent-solubilized cell extracts were purified by affinity chromatography on a lentil lectin column. Retained material was used in immune precipitation experiments following elution with alpha-methyl-D-mannoside.

Peptide analysis. Proteolytic digests of radiolabeled antigens identified by anti-CALLA MoAb on NALM-6 cells and granulocytes were compared using a modification of the method of Cleveland et al. Nonreducing sample buffer was added to pellets of iodinated antigens purified by immune precipitation, followed by a three-minute incubation at 110°C. Immediately before electrophoresis, samples were treated with fresh solutions of individual proteases in the same sample buffer or sham treated for 30 minutes, all at 22°C. Proteolysis was terminated by addition of 2-mercaptopethanol to a final concentration of 5%, followed by a repeat three-minute
incubation at 110 °C. Three proteases were used: trypsin, chymotrypsin, and papain (Sigma Chemical Co, St Louis).

RESULTS

Incubation of purified leukemic cells with specific antibody at 37 °C produces a cell population with a reduced capacity to bind anti-CALLA MoAb (antigenic modulation or, more correctly, epitope modulation) (Fig 1). In contrast, incubation of purified granulocytes from humans (nine donors) or from Macaca fascicularis (three animals) with anti-CALLA MoAb under identical conditions does not modulate expression of the detected epitope. Titration studies indicate that supernates from treated samples of both ALL cells and granulocytes contain comparable amounts of anti-CALLA MoAb after incubation at 37 °C with the same control and anti-CALLA-1 MoAb on anti-CALLA treated cells (thin and thick solid curves, respectively); Samples are as follows: control and anti-CALLA-1 MoAb on sham-modulated cells (thin and thick solid curves, respectively); control and anti-CALLA-1 MoAb on anti-CALLA-1 treated cells (thin and thick dashed curves, respectively). The ability to bind anti-CALLA-1 MoAb after incubation at 37 °C with the same reagent is decreased on ALL cells but not on granulocytes.

Anti-CALLA MoAb identify a 100-kd glycoprotein on lymphoid cells metabolically labeled with 35S-methionine or surface labeled with 125I. In contrast, the molecular weight (mol wt) of the antigen identified by anti-CALLA MoAb on granulocytes from the same individual varies with the method of labeling used. Metabolic labeling with 35S-methionine yields a 90-kd antigen, whereas surface labeling with 125I identifies one of 110 kd (Figs 2 and 3). Although the mol wt of the antigen detected in biosynthetically labeled granulocyte preparations suggests that it is not made by contaminating lymphoid cells, it does not permit unequivocal identification of p90 as CALLA. Because p90 is not found on granulocytes that are surface labeled with 125I, it may not be present or exposed on the cell surface. Further work is necessary to study these two forms of the granulocyte antigen.

To assess the homology of their polypeptide structures, proteolytic digests of the antigens identified by anti-CALLA-1 MoAb on iodinated human granulocytes and

![Fig 1. Modulation of CALLA. Cells from the ALL cell line Laz 221 or purified granulocytes were incubated with comparable amounts of anti-CALLA-1 or an isotype-matched control MoAb for 16 hours at 37 °C (Panels A and B, respectively). They were then assayed for their ability to bind control or anti-CALLA-1 MoAb. Samples are as follows: control and anti-CALLA-1 MoAb on sham-modulated cells (thin and thick solid curves, respectively); control and anti-CALLA-1 MoAb on anti-CALLA-1 treated cells (thin and thick dashed curves, respectively). The ability to bind anti-CALLA-1 MoAb after incubation at 37 °C with the same reagent is decreased on ALL cells but not on granulocytes.](image)

ALL cells were analyzed by SDS-PAGE and visualized by autoradiography (Fig 3). Proteolysis of the antigen isolated from ALL cells produces multiple novel lower mol wt species. Why several of the cleavage products generated by these three proteases have similar mol wt (most notably those at approximately 75, 55, and 45 kd) is unclear.

Proteolysis of the granulocyte antigen reveals a different pattern. New radiolabeled bands are much less intense than those observed with the lymphocyte antigen. Although several new bands are seen with proteolysis of both antigens, others appear to be unique to the lymphocyte antigen (partic-

![Fig 2. Antigens detected by anti-CALLA-1 MoAb on ALL cells and granulocytes metabolically labeled with 35S-methionine. Samples are as follows: A, control MoAb on NALM-6 cells; B, anti-CALLA-1 MoAb on NALM-6 cells; C, control MoAb on granulocytes; D, anti-CALLA-1 MoAb on granulocytes; and E, 14C-labeled mol wt standards. Markers were purchased from New England Nuclear (Boston) and include phosphorylase B (97 kd), albumin (69 kd), ovalbumin (46 kd), and carbonic anhydrase (30 kd).](image)
Fig 3. Proteolytic digests of iodinated antigens isolated from ALL cells (NALM-6) and human granulocytes by anti-CALLA-1 MoAb. Each antigen was treated with both 1 and 5 μg of each protease. Samples are as follows: Panel (A): A. sham-treated ALL antigen; B. sham-treated granulocyte antigen; C and D. ALL antigen treated with trypsin; E and F. granulocyte antigen treated with trypsin; G and H. ALL antigen treated with chymotrypsin; I and J. granulocyte antigen treated with chymotrypsin. Panel (B): A. sham-treated ALL antigen, B. sham-treated granulocyte antigen; C and D. ALL antigen treated with papain; E and F. granulocyte antigen treated with papain; G and H. sham-treated granulocyte antigen. Note the differences in mol wt between untreated antigens isolated from these two cell populations.

that have unique behavioral and chemical properties. Whereas the antigen on both normal and malignant lymphoid cells is readily modulated by specific antibody, that on isolated granulocytes is not. The mol wt of the iodinated antigen on human granulocytes is higher than that on normal and malignant lymphoid cells and on the nonhematopoietic cells tested, indicating that previously observed differences were not due to individual variation. In contrast, the mol wt of metabolically labeled antigen is lower in granulocytes than in lymphoid cells. Finally, proteolytic digests of the two antigens produce unique but overlapping banding patterns on SDS-PAGE.

Failure of the granulocyte antigen to modulate might reflect differences in either the integration of CALLA into the cell membrane of granulocytes and lymphocytes or the relative capacities of the surface structures of these two cell types to support antigenic modulation. Alternatively, the antigens detected on lymphoid cells and granulocytes may share an epitope(s) with one another but represent distinct chemical species. Because we have as yet no clear evidence that the five anti-CALLA MoAb used in our studies detect different antigenic determinants, this latter possibility cannot be excluded.

Despite major differences in the antigens detected by anti-CALLA MoAb on ALL cells and granulocytes, it is still not known whether these antigens differ in their protein structures. The unique mol wt of these two antigens might well result from posttranslational modifications of a common peptide, such as the addition of carbohydrate residues. Because differential glycosylation could modify both the sites of proteolysis and the mol wt of individual proteolytic fragments, the degree of homology of the peptide sequences of such antigens would be difficult to evaluate. Although analysis of the molecules from these two cell types in the total absence of glycosylation might clarify their relationship, production of such completely carbohydrate-free antigens is a difficult task. Amino terminal sequence analysis of the molecules on these two cell types might better address this issue.

REFERENCES


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