Rapid Publication

Detection of the Common Acute Lymphoblastic Leukemia Antigen in the Serum of Leukemia Patients

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Abstract. The common acute lymphoblastic leukemia antigen (CALLA) has been detected in biological fluids using a radioimmunoassay based on the inhibition of binding of ^{125}I-labeled monoclonal anti-CALLA antibody to glutaraldehyde-fixed NALM-1 cells. With this assay, we showed first that CALLA was released in culture fluids from NALM-1 and Daudi cell lines but was absent from culture fluids from CALLA negative cell lines. Then, we found that the sera of 34 out of 42 patients (81%) with untreated common acute lymphoblastic leukemia (c-ALL) contained higher CALLA levels than any of the 42 serum samples from healthy controls. The specificity of these results was further demonstrated by testing in parallel the sera from 48 patients with CALLA negative leukemias, including 26 acute myeloid leukemia (AML), 12 T-cell acute lymphoblastic leukemia (T-ALL), and 10 acute undifferentiated leukemia (AUL). All of these sera gave negative results, except for one patient with AUL, who had a significantly elevated circulating CALLA level, and one patient with AML, who had a borderline CALLA level, 3 SD over the mean of the normal sera. Preliminary results suggest that circulating CALLA is associated with membrane fragments or vesicles, since the total CALLA antigenic activity was recovered in the pellet of the serum samples centrifuged at 100,000 *g*. In addition, the CALLA-positive pellets contained an enzyme considered as a membrane marker, 5′-nucleotidase. Evaluation of the clinical importance of repeated serum CALLA determinations for the monitoring of c-ALL patients deserves further investigation.

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Introduction

The common acute lymphoblastic leukemia antigen (CALLA)[1] has been described initially as a cell surface antigen present on leukemic blasts in patients with the common type of acute lymphoblastic leukemia (c-ALL) or with chronic myelocytic leukemia in lymphoid blast crisis (1–7). In addition, normal lymphocyte precursors appear to express CALLA, especially during bone marrow regeneration (8), but this antigen has not been detected on immature myeloid precursors (9). Outside the hematopoietic system, CALLA has been identified on the surface of tubular and glomerular fetal and adult kidney cells (10) as well as on glioma (11) and melanoma (12) cells. Biochemically, CALLA has been characterized as a single polypeptide chain with an approximate molecular weight of 100 kD (13).

In this work, we report for the first time the presence of CALLA in the serum of patients with c-ALL. The circulating CALLA was detected by a newly developed radioimmunoassay (RIA) based on the inhibition of binding of ^{125}I-labeled anti-CALLA monoclonal antibody (MAb) to glutaraldehyde-fixed

1. *Abbreviations used in this paper:* AML, acute myeloid leukemia; AUL, acute undifferentiated leukemia; c-ALL, common acute lymphoblastic leukemia; CALLA, common acute lymphoblastic leukemia antigen; MAb, monoclonal antibody; T-ALL, T-cell acute lymphoblastic leukemia.

NALM-1 cells (14). This MAb, designated A12, has been shown (15) to react with the same epitope as the known J5 MAb (16).

Methods

Patients and controls. Serum samples were obtained from 90 patients with leukemia before any treatment. The leukemia cases consisted of 42 patients with c-ALL (identified by the presence of CALLA and Ia-antigens on leukemic cells (17) as well as the absence of T-cell markers (18)), 26 patients with acute myeloid leukemia (AML) (identified by Sudan Black staining and naphtol AS-D chloroacetate esterase positivity), 12 patients with T-cell acute lymphoblastic leukemia (T-ALL) (identified by the formation of rosettes with sheep erythrocytes and/or the presence of T-cell markers defined by various MAb), and 10 patients with acute undifferentiated leukemia (AUL) (classified by the absence of known cytochemical or surface markers on poorly differentiated blast). Additional controls consisted of serum samples from 42 unselected healthy blood bank donors.

In all cases the serum was collected after blood coagulation, followed by centrifugation at 2,000 *g* for 15 min. In two cases of c-ALL with previously identified elevated serum CALLA levels, blood samples were collected into three tubes; one without anticoagulant, one with EDTA, and one with heparin. The tubes were stored at room temperature, and serum or plasma was taken from each tube 1, 24, and 48 h after blood collection (the centrifuged blood was mixed gently after each removal of plasma or serum samples).

Monoclonal antibody and target cells. The MAb A-12 used in the CALLA RIA was obtained from a fusion between lymphocytes of a BALB/c mouse immunized with NALM-1 cells and cells from the myeloma line P3-NSI/1-Ag4 (19). As previously reported (15), MAb A12 is of IgG_1 subclass and reacts with the same epitope as the first described MAb anti-CALLA termed J5 (16). MAb A-12 was purified from hybridoma ascitic fluid by ion exchange chromatography, and labeled with ^{125}I by the chloramine T method.

Exponentially growing NALM-1 cells were fixed by a 10-min incubation at 25°C in 0.12% glutaraldehyde. The fixed cells retained their antigenicity for at least 1 mo when stored at 4°C in 0.15 M phosphate-buffered saline, pH 7.4 (PBS), containing 5% fetal bovine serum and 0.02% azide.

RIA. 30–40% of ^{125}I-labeled MAb A-12 (50 nCi representing about 6 ng of protein) was cell-bound after a 5-h incubation with 5×10^4 glutaraldehyde-fixed NALM-1 cells at 4°C. This binding was quantitatively inhibited up to 95% by NP-40 extracts of NALM-1 cells but not by extracts from CALLA-negative lines as demonstrated by the standard inhibition curve and controls presented in Fig. 1 *A*.

In the routine RIA procedure, radiolabeled MAb A-12 diluted in 50 μl of PBS containing 1 mg/ml of bovine serum albumin was incubated for 16 h at 25°C with 50 μl of either 20 times concentrated culture fluid from the NALM-1 cell line or dilutions of patients' sera. 50,000 glutaraldehyde-fixed NALM-1 cells were then added for a further incubation of 16 h at 4°C. The cells were washed with PBS containing 1 mg/ml of bovine serum albumin, and the cell-bound radioactivity was measured in a gamma-counter. Dilutions of a reference serum from a patient with a high CALLA level as well as from a pool of serum from normal individuals were always tested in parallel to calibrate each series of new serum samples to be analyzed.

Determination of 5'-nucleotidase activity. Assay of 5'-nucleotidase (EC 3.1.3.5) was performed as previously described (20). Briefly, pellets obtained by a 100,000 *g* centrifugation of 1 ml of patient serum or 200 μl of the serum supernatant were resuspended in 4 ml of 50 mM Tris–HCl buffer, pH 7.5, containing 2 mM 5'-AMP and 8 mM $MgCl_2$, and incubated for 20 min at 37°C. The reaction was stopped by the addition of 0.8 ml of 30% ice-cold trichloroacetic acid. The released inorganic phosphate was measured colorimetrically at 820 nm on 2-ml aliquots.

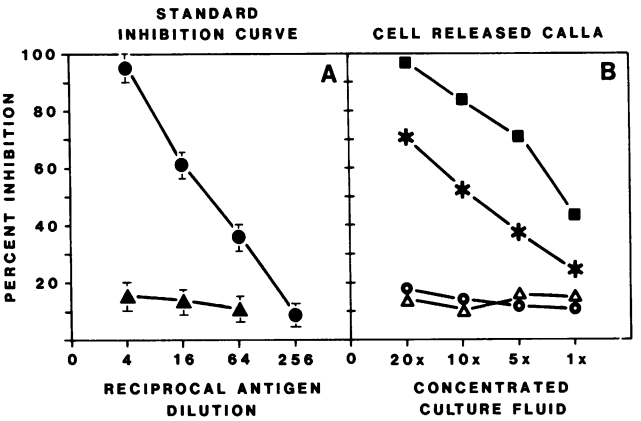

Figure 1. Inhibition of binding of ^{125}I-labeled monoclonal anti-CALLA antibody to glutaraldehyde-fixed NALM-1 cells by standard dilutions of NP-40 lysates (*A*) of NALM-1 cells (— ● —) or HL-60 cells (— ▲ —) as well as by concentrated culture fluid (*B*) from two CALLA-positive cell lines, NALM-1 (— ■ —) and Daudi (— • —), and two CALLA negative cell lines, HL-60 (— ○ —) and END-1 (— △ —).

Results

In a first series of experiments, the newly developed CALLA RIA was used to demonstrate the release of this antigen into culture fluids by two cell lines, NALM-1 (14) and Daudi (21), known to express CALLA on the cell surface. As shown in Fig. 1 *B*, 20 times concentrated culture fluids from NALM-1 and Daudi cell lines inhibited the CALLA RIA by 100 and 70%, respectively; whereas culture fluids from two CALLA-negative cell lines, the promyelocytic leukemia line HL-60 and the endometrial carcinoma line END-1, gave only background inhibition values of 15 and 18%.

Based on these results, the CALLA RIA was then used to search for CALLA in serum samples obtained from 90 patients with different types of acute leukemias. A serum sample from each patient was obtained before any treatment and assayed in duplicate at two different dilutions (1:1, 1:5). Individual mean inhibition values for undiluted serum samples from 42 cases of c-ALL, 12 cases of T-ALL, 26 cases of AML, 10 cases of AUL, and 42 normal controls tested in parallel are presented in Fig. 2. The mean inhibition for the control sera was 13.2% with a SD of 7.3.

We selected an arbitrary limit of 35% inhibition (3 SD over the mean inhibition value of the control sera) to operationally define positive and negative results. Under these

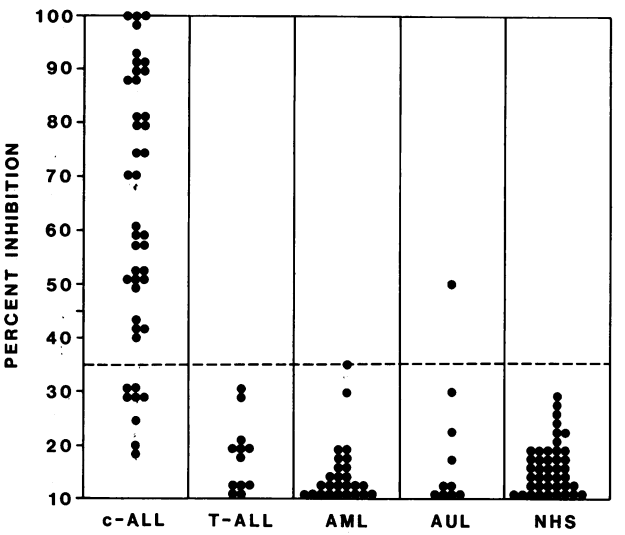

Figure 2. Individual serum CALLA levels from 42 patients with c-ALL, 12 with T-ALL, 26 with AML, 10 with AUL, and from 42 unselected normal blood donors. The results are expressed in percentages of inhibition of binding of ^{125}I-labeled monoclonal anti-CALLA antibody to NALM-1 cells.

conditions, 34/42 (81%) cases of c-ALL were positive, whereas none of the normal serum samples nor any of the 12 cases of T-ALL were positive. Only one of the 26 cases of AML gave a borderline inhibition, and only one of the 10 cases of AUL gave a significant inhibition of 51%.

Furthermore, sera from six patients with c-ALL were tested before treatment and after induction of complete remission. A marked drop of the CALLA values after treatment was observed in the four cases who had elevated values at presentation (Fig. 3).

In two c-ALL cases in which serum as well as EDTA and heparin plasma samples were obtained and separated from blood at different time intervals after blood collection (1, 24, and 48 h), we did not observe any difference in CALLA inhibition, neither between serum and plasma values nor between samples prepared by early or late centrifugations. These results suggest that the CALLA detected in serum or plasma has not been released from blood cells after blood collection.

Serum samples from six patients with elevated CALLA inhibition values were centrifuged at 100,000 *g* for 1 h at 4°C. The CALLA was measured by RIA both in the supernatant and the pellets. Virtually all of the CALLA inhibiting activity (95–100%) was found in the pellet fractions. In addition, pellet and supernatant fractions from CALLA-positive sera were tested for the presence of 5′-nucleotidase, an enzyme known to be associated with cell membrane (20). Again, 100% of the enzyme activity was detected in the pellet, with values ranging from 200 to 5,000 nmol/h per mg of protein. Together, these results suggest that the CALLA detected in the serum of c-ALL patients is associated with membrane fragments or vesicles.

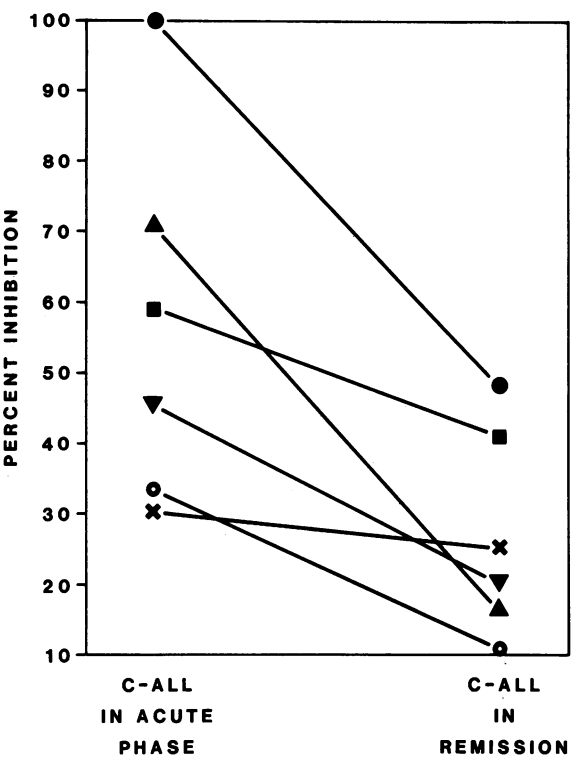

Figure 3. Comparison of serum CALLA levels before treatment and after clinical remission in six patients with c-ALL. Each line joins the two values from the same patient.

Discussion

In this context, it is of interest that a release of Ia antigens in the supernatant of cultured murine B-cell lymphomas has been observed (22). In this case also, the released antigen was recovered in the pellet after a centrifugation at 100,000 *g*.

The clinical significance of circulating CALLA remains to be ascertained. Along this line, it should be emphasized that all the cases of c-ALL tested here had been diagnosed by previous bone marrow examination and had >90% leukemia blasts. However, some of the cases had relatively low numbers of leukemic blasts in peripheral blood. There was no correlation between the CALLA levels in the serum and the number of malignant cells in the circulation. For instance, six cases with CALLA inhibition values ranging from 40 to 88% (mean = 53) had <5,000 blasts/μl of blood (mean = 1,900); whereas four cases with >20,000 blasts/μl of blood (mean = 37,800) had < 35% of CALLA inhibition value (mean = 24). This absence of correlation suggests that the major part of the CALLA material detected in the circulation is released by noncirculating leukemic cells. An alternative explanation could be that the blasts from each case of c-ALL have a different rate of CALLA release.

Prospective and sequential CALLA assays should be performed in the serum of patients with c-ALL in remission to

determine how early in the relapse this antigen becomes detectable in the circulation, and if sequential CALLA assays could substitute for some of the repeated bone marrow aspirations.

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