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Immunology

Molecular cloning of the common acute lymphoblastic leukemia antigen (CALLA) identifies a type II integral membrane protein

(cDNA/lymphoid progenitors/B-cell ontogeny)

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ABSTRACT **Common acute lymphoblastic leukemia antigen (CALLA) is a 100-kDa cell-surface glycoprotein expressed on most acute lymphoblastic leukemias and certain other immature lymphoid malignancies and on normal lymphoid progenitors. The latter are either uncommitted to B- or T-cell lineage or committed to only the earliest stages of B- or T-lymphocyte maturation. To elucidate the primary structure of CALLA, we purified the protein to homogeneity, obtained the NH_2-terminal sequence from both the intact protein and derived tryptic and V8 protease peptides and isolated CALLA cDNAs from a Nalm-6 cell line λgt10 library using redundant oligonucleotide probes. The CALLA cDNA sequence predicts a 750-amino acid integral membrane protein with a single 24-amino acid hydrophobic segment that could function as both a transmembrane region and a signal peptide. The COOH-terminal 700 amino acids, including six potential N-linked glycosylation sites compose the extracellular protein segment, whereas the 25 NH_2-terminal amino acids remaining after cleavage of the initiation methionine form the cytoplasmic tail. CALLA$^+$ cells contain CALLA transcripts of 2.7 to 5.7 kilobases with the major 5.7- and 3.7-kilobase mRNAs being preferentially expressed in specific cell types.**

Common acute lymphoblastic leukemia antigen (CALLA) was identified by heteroantisera and then by monoclonal antibodies (mAbs) as a 100-kDa cell-surface glycoprotein on most acute lymphoblastic leukemias (1, 2). Additional lymphoid malignancies including lymphoblastic, Burkitt, and nodular poorly differentiated lymphocytic lymphomas as well as chronic myelogenous leukemias in lymphoid blast crisis express CALLA (3). In contrast, acute myelogenous leukemias and mature B- and T-cell lymphomas lack the antigen (3).

Originally thought to be a tumor-specific antigen, CALLA was later identified on early lymphoid progenitor cells within bone marrow and fetal liver (3–5). These CALLA$^+$ cells have phenotypic markers of a population either uncommitted to B- or T-cell lineage or committed to only the earliest stages of B-cell differentiation. CALLA is also expressed on fetal and pediatric thymocytes with the structural and phenotypic markers of very early T-cell precursors (3, 6, 7). The selective expression of CALLA in lymphoid malignancies is thought to reflect the restricted expression of the antigen during the earliest stages of normal lymphoid differentiation.

Although antibodies against CALLA are used extensively in diagnosis and therapy of lymphoid malignancies (3, 8), the primary structure and function of CALLA remain unknown. To further define this important cell-surface structure in molecular terms, we obtained amino acid sequence from the purified CALLA protein and isolated the full-length CALLA cDNA.‖

MATERIALS AND METHODS

Immunoprecipitation, Protein Purification, and Microsequencing. Nalm-6 cells (3×10^7) were radioiodinated using lactoperoxidase and lysed in radioimmunoprecipitation assay (RIPA) buffer (9) containing 1% Triton X-100 (TX-100), 0.15 M NaCl, 1 mM phenylmethylsulfonyl fluoride, 80 mM iodoacetamide, trypsin inhibitor at 0.02 μg/ml, and chymostatin, leupeptin, pepstatin, and antipain each at 0.5 μg/ml. Ultracentrifuged lysates were precleared sequentially with preimmune rabbit immunoglobulin and mouse mAb anti-β2 microglobulin coupled to protein A–Sepharose CL-4B, and CALLA was immunoprecipitated with the mouse mAb J5 (3) coupled to protein A–Sepharose. The anti-CALLA beads were washed with (*i*) 10 mM Tris, pH 8/0.15 M NaCl (Tris-buffered saline, TBS)/1% deoxycholate, (*ii*) TBS/1% deoxycholate/0.05% $NaDodSO_4$, and (*iii*) TBS/1% Nonidet P-40. Bound CALLA was eluted with $NaDodSO_4$ sample buffer containing 5% (vol/vol) 2-mercaptoethanol. Endoglycosidase-F treatment was accomplished as described (10), and aliquots of samples were analyzed with and without enzyme treatment on 10% $NaDodSO_4$/PAGE.

For preparative isolation of the CALLA protein, 10^{11} Nalm-6 cells were lysed for 1 hr at 4°C in 400 ml of lysis buffer. Affinity purification of CALLA was completed as described (10) with mAb J5. Purified CALLA protein was then analyzed for NH_2-terminal sequence on a gas-phase protein sequenator (Applied Biosystems, Foster City, CA; model 470A equipped with an on-line 120A phenylthiohydantoin analyzer using program 03RPTH).

Protein Fragmentation. Four hundred picomoles of electroeluted CALLA in 50 mM NH_4HCO_3/0.1% $NaDodSO_4$ was mixed with V8 protease (Boehringer Mannheim) to give a protein/enzyme ratio of 5:1. After incubation at 37°C for 1 hr and then at 22°C for 16 hr, the sample was made 0.1% in trifluoroacetic acid and applied to a reverse-phase C_{18} HPLC column to separate fragments as described below.

One nanomole of electroeluted CALLA was made 0.1 M Tris·HCl, pH 8/20 mM dithiothreitol/2% (wt/vol) $NaDodSO_4$ and adjusted 60 min later to 50 mM in iodoacetic acid. The reduced S-carboxymethylated preparation was then precipitated by addition of 9 vol of ethanol at −20°C for 16 hr, dissolved in 0.1 M Tris·HCl, pH 8/2 mM $CaCl_2$, mixed with tosylphenylalanine chloromethyl ketone-treated (TPCK) trypsin (Cooper) to give a protein/enzyme ratio of 50:1, and

Abbreviations: CALLA, common acute lymphoblastic leukemia antigen; mAb, monoclonal antibody; TBS, Tris-buffered saline.

§Deceased, January 24, 1988.

‖The sequence reported in this paper is being deposited in the EMBL/GenBank data base (Intelligenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03779).

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incubated for 16 hr at 37°C. After addition of trifluoroacetic acid to 0.1% and microcentrifuging, the supernatant was subjected to reverse-phase HPLC (Vydak, TP54, 4.6 mm × 25 cm, 5-μm C_{18}) at 1 ml/min, eluting in 0.1% trifluoroacetic acid, using a HP1090 chromatograph equipped with a diode-array detector (Hewlett–Packard). A 0–50% acetonitrile gradient over 50 min was produced, and elution fractions of 1 ml were collected. Selected fractions were further HPLC-purified using an acetonitrile gradient of 25–45% over 15 min with a flow rate of 1 ml/min and collection of 0.5-ml fractions. V8 protease fragments and tryptic peptides were then analyzed for NH_2-terminal sequence.

cDNA Library Construction and Screening and DNA Sequencing. Nalm-6 cell line RNA was selected with oligo(dT) twice and used to construct a λgt10 cDNA library (11, 12). Redundant oligonucleotide probes were synthesized on an Applied Biosystems model 381A DNA synthesizer, labeled at their 5′ ends, and used to screen the Nalm-6 cDNA library. Filters were hybridized with the appropriate oligonucleotide probe for 16 hr in 6× standard saline citrate (SSC) (1× SSC = 0.15 M sodium chloride/0.015 M sodium citrate, pH 7)/5× Denhardt's solution (1× Denhardt's solution = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin)/single-stranded DNA at 10 μg/ml/0.1% $NaDodSO_4$/0.05% NaP_iPO_4 and washed in 6× SSC/0.1% $NaDodSO_4$. Hybridization and final wash temperatures were 44°C and 48°C for oligonucleotide 1, 26°C and 30°C for oligonucleotide 2, and 38°C and 42°C for oligonucleotide 3.

Individual *Eco*RI inserts from clones 1.1 and 1.2 were subcloned into the M13 vector mp18 for sequencing by the Sanger dideoxy chain-termination method (13); the universal M13 primer and sequence-specific oligonucleotide primers were used. *Bgl* II/*Hin*dIII fragments from clone 1.2 were also subcloned into mp18 and sequenced to confirm the orientation and positions of individual *Eco*RI fragments. Clones 2.1, 2.2, 2.3, and 3.1 were subcloned and sequenced as above. All sequences were determined in both directions.

RNA Analysis. RNA samples were prepared and analyzed by blotting (14). Filters were hybridized with a ^{32}P-labeled 1587-bp *Eco*RI CALLA cDNA fragment, which includes bp 541–2227 (Fig. 2*C*). Filters were washed in 2× SSC/0.1% $NaDodSO_4$/PAGE at 25°C for 30 min and 65°C for 30 min and then in 0.2× SSC/0.1% $NaDodSO_4$ at 65°C for 60 min. Cell lines used included Nalm-6, Raji, MOLT-4, HSB, Jurkat, and J77 (from laboratory stocks) and HB-100, CCL-227, and G-361 (gifts from L. B. Chen and M. Wick, Dana–Farber Cancer Institute).

RESULTS AND DISCUSSION

Biochemical Characterization, Purification, and Microsequencing of the CALLA Protein. To isolate CALLA protein, we used the Nalm-6 line as a cellular source in conjunction with the anti-CALLA mAb J5 (2). The Nalm-6 cell line was originally derived from a patient with chronic myelogenous leukemia in lymphoid blast crisis and expresses high levels of surface CALLA (15). Fig. 1*A* (lane a) shows that mAb J5 immunoprecipitates a structure of 97- to 100-kDa from ^{125}I-surface-labeled Nalm-6 cells. After digestion with endoglycosidase F, molecular mass of CALLA decreases by ≈10 kDa, indicating at least 10% of the molecular mass of CALLA results from N-linked carbohydrates (Fig. 1*A*, lane b and ref. 16). Two-dimensional electrophoresis shows CALLA protein to migrate as a single polypeptide exhibiting limited microheterogeneity (data not shown and ref. 16). Purified CALLA was obtained by passing detergent lysates from 10^{11} Nalm-6 cells over an immunoaffinity column followed by preparative $NaDodSO_4$/PAGE as described (10). Silver staining of an aliquot of the purified CALLA protein on $NaDodSO_4$/PAGE indicates the purity of sample preparations (Fig. 1*A*, lane c).

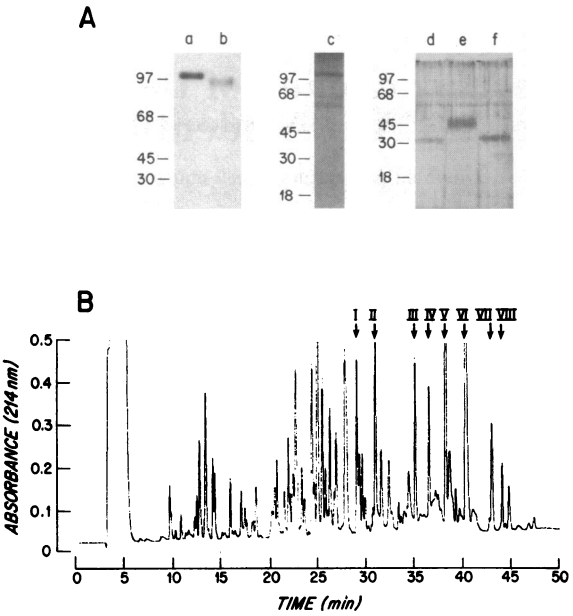

FIG. 1. (*A*) Analysis of purified CALLA and V8 protease-derived fragments. Surface-radioiodinated CALLA was immunoprecipitated with mAb J5 and analyzed on $NaDodSO_4$/PAGE without (lane a) or with (lane b) endoglycosidase-F digestion. An aliquot of $NaDodSO_4$/PAGE-separated CALLA and each purified V8 protease-derived fragment (I–III) used for protein sequencing are shown after silver staining in lanes c and d–f, respectively. (*B*) HPLC profile of a CALLA tryptic digest. Roman numerals indicate peaks selected for further purification and NH_2-terminal sequencing.

Fifty picomoles of purified CALLA were subjected to NH_2-terminal sequencing. However, only a 5-pmol signal (XXSESQ) was obtained, suggesting that CALLA was in large part NH_2-terminally blocked to Edman degradation. Therefore, CALLA was digested with V8 protease, and the resulting fragments were separated by reverse-phase HPLC and examined by $NaDodSO_4$/PAGE. Three pure V8 fragments (Fig. 1*A*, lanes d–f) were identified and sequenced giving the residues indicated in Fig. 2*C*. Of note, V8 peptide III contains sequence identical to that from intact CALLA protein (*XXSESQ*MDITDINTP), indicating that V8 peptide III is derived from the partially blocked NH_2 terminus. A second preparation of CALLA was reduced, S-carboxymethylated, and digested to completion with tosylphenylalanine chloromethyl ketone-treated (TPCK) trypsin. Reverse-phase HPLC separation of the resulting tryptic digest yielded at least 80 different peaks (Fig. 1*B*); eight of these were selected for further HPLC purification with an alternative gradient yielding eleven tryptic peptides with sequences shown in Fig. 2*C*. Interestingly, tryptic peptide VIII and protease V8 peptide I contain an overlapping amino acid sequence LNYKEDEYFENIIQN. Cleavage at the lysine residue in tryptic peptide VIII probably did not occur because tryptic digestion at lysine residues followed by COOH-terminal acidic amino acids is sometimes ineffective (17).

Isolation and Sequencing of CALLA cDNAs. To isolate cDNA clones encoding CALLA, redundant oligonucleotide probes corresponding to amino acid sequences from the tryptic and V8 peptides were used to screen a Nalm-6 λgt10 cDNA library. Oligonucleotide 1 (3′ TTY-CTY-CTR-CTY-ATR-AAR-CTY-TT 5′, Y = T or C, R = A or G), which corresponds to eight amino acids in both tryptic peptide VIII and V8-I (Fig. 2*C*) was used in initial screening. Positive clones were rescreened with a second oligonucleotide (3′ TTR-TTR-CTY-ATR-RAN-CT 5′, N = A, T, C, or G), which corresponds to six amino acid residues NH_2-terminal to those

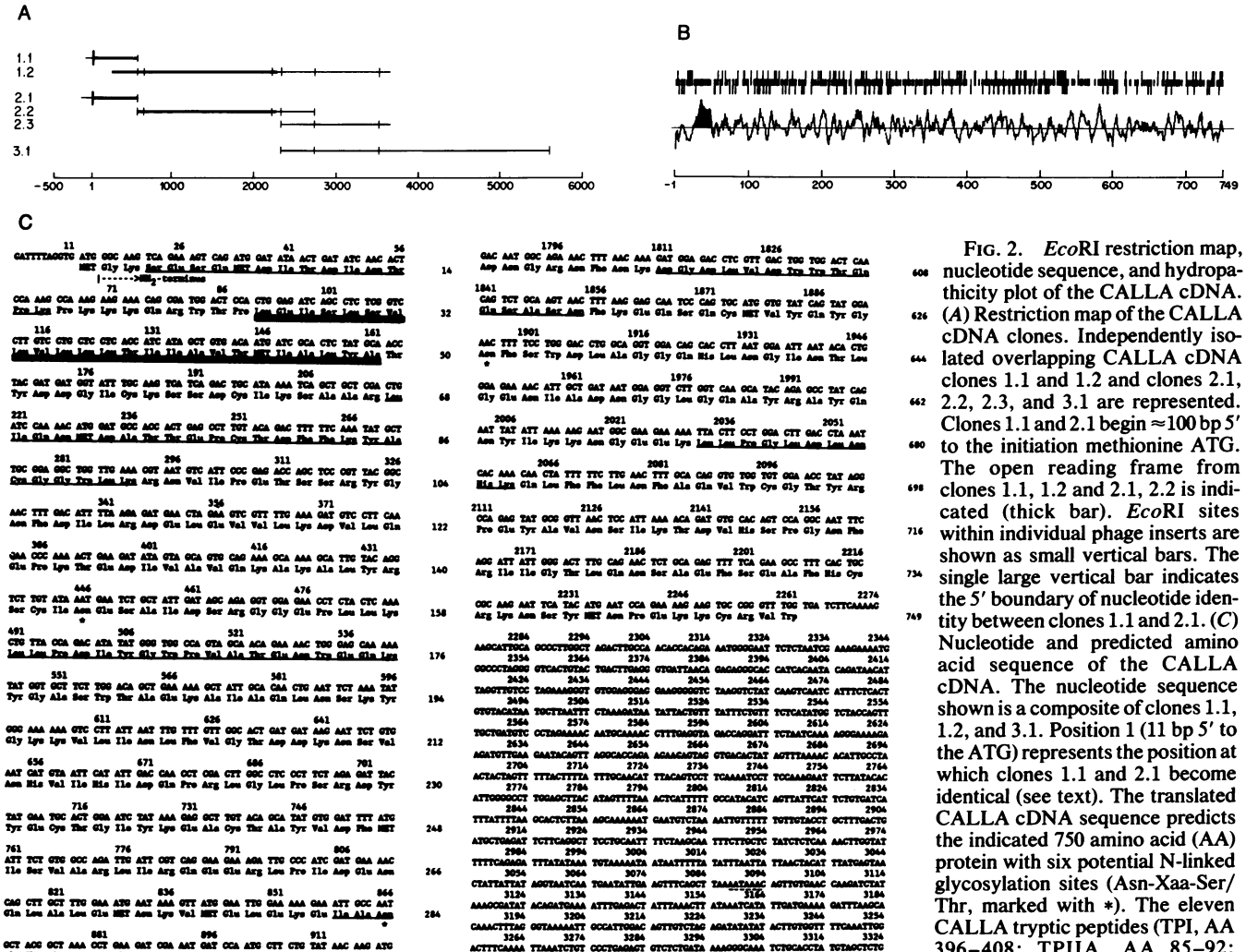

FIG. 2. *Eco*RI restriction map, nucleotide sequence, and hydropathicity plot of the CALLA cDNA. (*A*) Restriction map of the CALLA cDNA clones. Independently isolated overlapping CALLA cDNA clones 1.1 and 1.2 and clones 2.1, 2.2, 2.3, and 3.1 are represented. Clones 1.1 and 2.1 begin ≈100 bp 5′ to the initiation methionine ATG. The open reading frame from clones 1.1, 1.2 and 2.1, 2.2 is indicated (thick bar). *Eco*RI sites within individual phage inserts are shown as small vertical bars. The single large vertical bar indicates the 5′ boundary of nucleotide identity between clones 1.1 and 2.1. (*C*) Nucleotide and predicted amino acid sequence of the CALLA cDNA. The nucleotide sequence shown is a composite of clones 1.1, 1.2, and 3.1. Position 1 (11 bp 5′ to the ATG) represents the position at which clones 1.1 and 2.1 become identical (see text). The translated CALLA cDNA sequence predicts the indicated 750 amino acid (AA) protein with six potential N-linked glycosylation sites (Asn-Xaa-Ser/Thr, marked with *). The eleven CALLA tryptic peptides (TPI, AA 396–408; TPIIA, AA 85–92; TPIIB, AA 425–436; TPIIC, AA 159–176; TPIII, AA 68–84; TPIVA, AA 365–372; TPIVB, AA 437–446; TPV, AA 599–613; TPVI, AA 673–682; TPVII, AA 448–464; and TPVIII, AA 492–514) and the three CALLA V8 peptides (V8I, AA 499–514; V8II, AA 282–300; and V8III, AA 3–16) identified by protein microsequencing are underlined in the translated CALLA cDNA sequence. The single 24-amino acid hydrophobic segment (amino acids 27–50) is underlined with a thick bar. Polyadenylylation signals (AATAAA) are underlined with a dotted line. (*B*) Hydropathicity plot. Top line shows charge distribution for the predicted CALLA protein sequences (up = positive, down = negative, flat = no change). Middle line shows hydrophobicity values calculated by the PRSTRC algorithm assigned over windows of five amino acids (18). Positions above the baseline are hydrophobic. Bottom line gives amino acid position numbers (−1 is the cleaved initiation methionine). The transmembrane segment is shaded.

of oligonucleotide 1 (Fig. 2*C*). cDNA inserts from clones containing sequences complementary to both probes were further analyzed. Fragments from the clone containing the longest cDNA insert [3537 base pairs (bp)] were subcloned

into the m13 vector mp18 and sequenced by the Sanger dideoxy chain-termination method. This cDNA [clone 1.2 (Fig. 2*A*); bp 199–3734 (Fig. 2*C*)] sequence has an open reading frame that contains all eleven tryptic peptides but only two of the three V8 peptides determined by microsequencing of CALLA protein (Fig. 2 *A* and *C*). The clone 1.2-translated cDNA sequence lacks the NH_2-terminal residues identified from intact CALLA protein and V8 peptide III (Fig. 2*C*). An overlapping cDNA (clone 1.1) that contains the complete NH_2 terminus was identified by rescreening the Nalm-6 cDNA library with an oligonucleotide (3′ GTY-TAC-CTR-TAD-TGN-CTR-TA 5′, D = A, G, or T) corresponding to seven amino acids from V8 peptide III (Fig. 2*C*). To confirm the CALLA cDNA sequence obtained from clones 1.1 and 1.2, a second set of overlapping CALLA cDNA clones (2.1, 2.2, and 2.3, Fig. 2*A*) was characterized. The independently derived CALLA cDNA sequences from clones 1.1 and 1.2 and from 2.1, 2.2, and 2.3 are identical from bp 1 to 3734, including 11 nucleotides of 5′ untranslated sequence. However, each clone contains an additional unique ≈100 bp of 5′ sequence (data not shown); this difference could represent alternative 5′ splicing. An additional overlapping clone, 3.1, was identified that corresponds to the previously characterized clones from its 5′ end (bp 2321) through bp 3733; thereafter, clone 3.1 contains an additional 1775 bp of 3′ untranslated sequence that ends in a poly(A) tail (Fig. 2 *A* and *C*).

The complete nucleotide sequence of the CALLA cDNA as deduced from clones 1.1, 1.2, and 3.1 contains an open reading frame of 2250 bp (positions 12–2261) beginning with an ATG methionine, which is preceded by an in-frame TAG termination codon at bp 6–8. After the open reading frame, there is a TGA termination codon at positions 2262–2264 and 3244 bp of 3′ untranslated sequence ending in a poly(A) tail at bp 5508. Canonical polyadenylylation signals (AATAAA) are also found at positions 3088, 3371, 3792, 4406, and 5484 (Fig. 2*C*). Clones 1.2 and 2.3 have poly(A) sequences of 11 and 18 bp, respectively (starting at bp 3723), but do not contain a discernible polyadenylylation signal within 50 bp 5′ of the sequence of adenines. As clone 3.1 has a sequence of 10 adenines in the same location, clones 1.2 and 2.3 probably result from internal priming by oligo(dT) at this poly(A) sequence in CALLA mRNA.

Primary Structure of the CALLA Protein. The translated CALLA cDNA sequence includes 178 of the 182 amino acid residues identified by microsequencing the intact CALLA protein, the derived tryptic peptides, and the V8 protease fragments (Fig. 2*C*), providing conclusive evidence that the cDNA represents authentic CALLA. The CALLA cDNA predicts a 750 amino acid protein with a polypeptide core of 85.5 kDa and six potential N-linked glycosylation sites (Asn-Xaa-Ser or Thr) located at amino acid positions 144, 284, 310, 324, 334, and 627. The fact that endoglycosidase F digestion decreases the molecular mass of CALLA by only 10 kDa (Fig. 1*A*, lane b) suggests that all potential glycosylation sites are not used in Nalm-6 cells. The molecular mass of CALLA after removal of N-linked sugars is consistent with the core-protein size predicted from the CALLA cDNA (Figs. 1 and 2).

The translated CALLA cDNA sequence has a single hydrophobic 24 amino acid segment at positions 27–50 with characteristics of a transmembrane region (Fig. 2*B*). This hydrophobic segment is preceded by five basic residues, suggesting that CALLA is oriented such that the NH_2 terminus constitutes the cytoplasmic tail. The translated CALLA sequence does not contain an initial hydrophobic NH_2-terminal segment that could function as a signal peptide. In fact, comparison of the translated cDNA sequence with that derived from the intact protein and NH_2-terminal V8 fragment (V8-III) reveals that the only NH_2-terminal proteolytic processing that occurs during CALLA synthesis removes the initiation methionine (Fig. 2*C*). In contrast to studies (16) suggesting that CALLA is a nonintegral membrane protein, our results show CALLA to be a type-II integral membrane protein (19) with a short (25 amino acid) NH_2-terminal cytoplasmic tail, a single 24 amino acid hydrophobic region that functions as an uncleaved internal signal sequence and a transmembrane segment, and a large (700 amino acid) COOH-terminal extracellular domain. That all six putative N-linked glycosylation sites are located in the COOH-terminal segment agrees with this interpretation as does the fact that CALLA can be radioiodinated on the cell surface given that no tyrosine residues exist in the NH_2-terminal segment (Figs. 1*A* and 2*C*).

Searches of the GenBank data base (release 52.0) and data base from the Protein Identification Resource (release 12.0) reveal that CALLA is unrelated to known proteins and has no significant internal duplications or previously characterized active sites or consensus binding-site sequences. The NH_2-terminal region immediately preceding and including the transmembrane segment of CALLA has partial identity (14 of 31 amino acids with no gaps) with that of another type-II membrane protein, prosucrase-isomaltase (20), raising the interesting possibility that the dual-function transmembrane segments of certain type-II proteins have common features. Posttranslational cleavage of NH_2-terminal methionine residues has also been described in other type-II membrane proteins (20, 21).

RNA Analysis of CALLA: Multiple Related CALLA Transcripts. RNAs from a panel of $CALLA^+$ and $CALLA^-$ lymphoid cell lines and primary tumors were probed in RNA analysis with a 1587-bp *Eco*RI fragment from the 1.2 CALLA cDNA clone. Cell lines defined as $CALLA^+$ (by immunofluorescence using mAb J5) including Nalm-6, the acute lymphoblastic leukemia cell lines Laz 221 and MOLT-4, and the Burkitt lymphoma cell line Raji contain two major CALLA mRNAs, of 3.7 kb and 5.7 kb (Fig. 3*A*). In contrast, $CALLA^-$ sources including three T-cell tumor lines—Jurkat, J77 (a second Jurkat clone), and HSB, an Epstein–Barr virus-transformed lymphoblastoid line—Laz 388, and resting and mitogen-stimulated peripheral blood lymphocytes lack these transcripts (Fig. 3*A*). Additional low-abundance CALLA transcripts also occur in $J5^+$ cells. For example, in poly(A)$^+$ RNA from Nalm-6, low abundance CALLA-related mRNAs of 4.5, 3.1, and 2.7 kb are also detected by RNA blotting (Fig. 3*A*, lane 11). Probably the 5.7-kb transcript results from use of the downstream canonical polyadenylylation site located in clone 3.1 because an

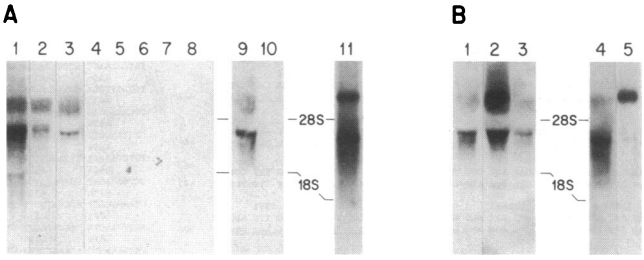

FIG. 3. RNA blot hybridization analysis. (*A*) RNA analysis of lymphoid cells. Twenty micrograms of total RNA from the following cell types was analyzed: Nalm-6 (lane 1); Raji (lane 2); MOLT-4 (lane 3); HSB, Jurkat, and J77 (lanes 4–6); unstimulated peripheral blood mononuclear cells (lanes 7); mitogen-triggered PBMC (lane 8); Laz 221 (lane 9); and Laz 388 (lane 10). Ten micrograms of poly(A)$^+$ selected Nalm-6 RNA was also analyzed (lane 11). (*B*) RNA analysis of nonlymphoid cells. Twenty micrograms of total RNA from the following cell types was analyzed: HB-100 (lane 1); G361 (lane 2); CLL-227 (lane 3); Nalm-6 (for comparison, lane 4); and granulocytes (lane 5).

antisense oligonucleotide derived from the 3'-untranslated region unique to clone 3.1 (bp 4865–4903) identifies only the larger 5.7-kb transcript. The 3.7-kb transcript probably results from use of the polyadenylylation signal at bp 3792, and the minor mRNAs of 4.5, 3.1, and 2.7 kb from use of polyadenylylation signals located at 4406–4411, 3371–3376, and 3088–3093, respectively.

Anti-CALLA mAb reactivity has also been seen on normal granulocytes, a subpopulation of bone-marrow stromal cells, certain elements in kidney, fetal intestine, breast and some nonlymphoid tumor cells (22–25). Except for granulocyte CALLA protein, which peptide mapping shows to be related to that from lymphoid cells (26), the basis of anti-CALLA reactivity with these other nonlymphoid sources is unknown. To determine whether CALLA transcripts from nonlymphoid sources resemble those in lymphoid cells, total RNA was isolated from granulocytes, a CALLA$^+$ fibroblast strain (HB-100) and two additional CALLA$^+$ tumor cell lines, a melanoma line (G-361), and a colon carcinoma line (CLL-227) (24) and probed by RNA blot. As in Nalm-6 cells, the 3.7-kb transcript is the major CALLA mRNA in HB-100 and CCL-227 cells. In contrast, the 3.7- and 5.7-kb CALLA transcripts are both abundant in G-361 cells, whereas the 5.7-kb mRNA is the major CALLA transcript in granulocytes (Fig. 3*B*). This tissue-specific differential expression of the 3.7- and 5.7-kb CALLA transcripts in different CALLA$^+$ cell types is presently unexplained but may result from interaction of 3'-untranslated-region regulatory elements with tissue-specific factors (26). Therefore, we note that the CALLA 3'-untranslated region contains several A + T-rich regions containing the ATTTA motif recently associated with instability of certain transiently expressed mRNAs (bp 2984–3028, 3144–3181, and 4344–4362) (27). Whether these sequences mediate the stability of CALLA mRNAs in specific cell types remains to be determined. Nevertheless, the expression of the same major CALLA messages in these nonlymphoid sources indicates that mAb J5 recognizes authentic CALLA on these cells and not another protein with a common J5 epitope. Presumably, the small differences in sizes of lymphoid and granulocyte CALLA proteins (95- to 100-kDa versus 95- to 110-kDa) (26) relate to different patterns of glycosylation.

Implications. Molecular cloning of CALLA and its identification as a type-II transmembrane glycoprotein do not allow inference of its role in lymphoid function or differentiation. Proteins in this class have diverse functions ranging from receptors to membrane-bound enzymes and include the transferrin receptor, the asialoglycoprotein receptor, influenza viral neuraminidase, γ glutamyl transpeptidase, prosucrase–isomaltase complex, and the invariant chain of HLA (19).

That CALLA is an integral membrane protein is consistent with previous studies showing rapid cell-surface redistribution and internalization and degradation of the CALLA–antibody complex after specific antibody treatment of CALLA$^+$ cells at 37°C (22, 24, 28). Antibody-induced modulation of CALLA resembles that seen with cell-surface receptors, such as surface immunoglobulin and T3–Ti (29, 30); this modulation also resembles the specific downregulation or loss of cell-surface receptors induced by many peptide hormones (31, 32). That CALLA has a relatively short cytoplasmic tail argues against a direct signal-transduction function for it.

CALLA expression appears on uncommitted TdT$^+$ lymphoid progenitors and generally declines as the cells display evidence of B-cell (cμ) or T-cell (T11) commitment (5, 6), suggesting that the antigen may function in the earliest stages of lymphoid differentiation. That CALLA is also expressed by a subpopulation of bone-marrow stromal cells (23) raises the interesting possibility that CALLA participates in the hematopoietic microenvironment necessary for early lymphoid maturation (possibly by means of adhesion, homing, or motility). The molecular characterization of CALLA allows further study of its role in normal and neoplastic lymphoid differentiation.

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