Characterization of a New T-Lineage Glycoprotein Expressed in Mature T-Cell Leukemias and Lymphomas

By William F. Cassano

We identified a new human, T-lineage restricted glycoprotein of molecular weight 120 Kd that is expressed primarily in mature T-cell malignancies. The antigen, named TCA-1 (T-cell cytoplasmic antigen), is an intracellular glycoprotein found mainly in the Golgi stacks, although a few cell lines also display surface membrane TCA-1. Many but not all T-cell neoplasms express this antigen. The antigen is absent from neoplastic and normal human tissue outside the T-lymphocyte lineage. TCA-1 was identified by murine monoclonal antibodies produced after immunization of mice with T-cell chronic lymphocytic leukemia cells. The glycoprotein is a monomer containing approximately 4% N-linked carbohydrate with terminal D-galactose residues. Partial amino acid sequence analysis of TCA-1 shows homology with an immunoglobulin heavy chain region, which suggests that TCA-1 may belong to the immunoglobulin supergene family of receptor and adhesion molecules.

ADVANCES IN the identification of lymphoblasts of T-cell lineage through immunophenotyping with a wide array of monoclonal antibodies (MoAbs) to lymphohematopoietic cell antigens have led to improved understanding of the biology of T-cell neoplasia. The distinct clinical entities of mediastinal and cutaneous lymphomas are found to be neoplasms of T-cell origin. T-lineage, acute lymphoblastic leukemia (ALL) is distinguished by high tumor burdens at diagnosis and a poor prognosis. The HTLV-1 virus-associated lymphomas seen in Japan, the Caribbean, and southeastern United States are transformed T cells expressing a T-cell lineage through immunophenotyping with a wide array of monoclonal antibodies (MoAbs) to lymphohematopoietic cell antigens.

The catalog of T-cell-related antigens identified by MoAbs continues to grow rapidly. The initial identification of the thymocyte antigen CD1; the pan-T cell antigens CD2, CD3, CD5, CD6, and CD7; the inducer suppressor subset markers CD4 and CD8; and the T-cell activation antigen (and growth factor receptor) CD25 provided useful reagents for immunophenotyping lymphomas and leukemias. While much of the early progress in defining T-lineage neoplasms depended on these reagents, it soon became evident that many of these antigens were also present on neural and other cells not of lymphohematopoietic origin. Although important, functional roles in regulating the immune response have been attributed to many of these antigens, none are related to the neoplastic transformation of T lymphocytes. Thus, antigens whose expression is strictly confined to T cells are still quite rare, and antigens unique to malignant transformed T cells are unknown, except for the HTLV-D derived antigens.

We now present data characterizing a new, glycoprotein antigen called TCA-1 (T-cell cytoplasmic antigen), whose expression is limited exclusively to T cells, with the highest levels of antigen being found in T-cell leukemias and lymphomas.

MATERIALS AND METHODS

Cell lines. Human hematopoietic cell lines, obtained from and verified by Dr. P. Martin as previously described, 1 were maintained in RPMI 1640 and 12% fetal calf serum (FCS), supplemented with glutamine and pyruvate. Cultures were split every 3 days to maintain logarithmic growth. Circulating neoplastic cells from a patient with T-cell chronic lymphocytic leukemia (T-CLL) (CD2+, CD5+, CD7+, CD20+, HLA-DR+) (designated SHB) were collected by leukapheresis and cryopreserved in liquid nitrogen. A spontaneous lymphoblastoid cell line, shown to be of B-cell phenotype and designated SHB, was generated from peripheral blood from this same patient after 6 weeks in culture. Surface phenotyping confirmed a CD20+, HLA-DR+, mature B-cell pattern.

Blood cell separations. Peripheral blood mononuclear cells (PBMCs) were obtained from heparinized whole blood by density gradient centrifugation over Ficol-Hypaque (d = 1.077). Twenty milliliters of heparinized whole blood (50 U heparin/mL) was diluted with an equal volume of RPMI 1640 in a 50-mL sterile centrifuge tube. The blood was then carefully overlaid with 10 mL of Ficol-Hypaque and centrifuged at 600 x g for 20 minutes. The buoyant cell layer was then aspirated, diluted with RPMI 1640, and centrifuged at 400 x g for 10 minutes. Finally, the supernatant was discarded, the cell pellet resuspended in RPMI 1640, and centrifuged at 300 x g for 6 minutes. The supernatant was discarded and the cell pellet resuspended in a convenient volume of RPMI 1640 for cell counting.

T-cell clones. Antigen nonspecific T-cell clones were isolated by limiting dilution cloning of phytohemagglutinin (PHA) activated, human T cells. First, the purified T-cell fraction of mononuclear cells prepared as described above was suspended in RPMI 1640 with 12% FCS and 1 µg/mL PHA (Burroughs-Wellcome, Research Triangle Park, NC) at 10⁶ cells/mL. After 4 days the PHA-activated T-cell blasts (PHA blasts) were unclumped by repeated pipetting and counted. Three hundred cells were removed and placed into a tube along with 3 x 10⁶ recombinant IL-2 (rIL-2) (Ampgen, Thousand Oaks, CA), along with 3 x 10⁶ irradiated (2,000 cGy) mononuclear cells. The 30-mL mixture was then distributed into three 96-well flat-bottom plates containing 100 µL/well. Wells were refed with fresh media, PHA, IL-2, and irradiated feeder cells every 7 days. A cloning efficiency of 30% to 50% in short-term cultures was achieved. Selected vigorously growing wells were recloned by limiting dilution to ensure monoclonality, expanded in bulk culture, and phenotyped with CD4 and CD8 MoAbs.

MoAb production. Antibody WC1.1, a murine monoclonal immunoglobulin (lg) M, was produced by intraperitoneal immunization of BALB/c mice with 1 x 10⁶ SH cells four times during a

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3-week period. Three days after the final immunization, the mouse was killed, the spleen removed, and a single cell suspension prepared by crushing between sterile, etched glass slides. The fusion of 150 to 250 x 10^6 spleen cells and 50 to 10^6 NS-1 myeloma cells was performed with 1.5 mL of 40% polyethylene glycol 1450 (adjusted to pH 8.0 immediately before use) for 6 minutes, followed by slow dilution over 3 minutes with 10 mL RPMI 1640. Cells were then pelleted; resuspended in RPMI 1640 with 12% FCS, supplemental glutamine, pyruvate, and hypoxanthine-aminopterin-thymidine (HAT) (Sigma, St Louis, MO); and distributed into four 96-well flat-bottomed 48-well plates containing 200 μL/well. Successful hybrids selected by growth in HAT medium for 10 days were screened for production of antibody, which bound to SH or Jurkat cells but not normal PBMCs, using an indirect immunoperoxidase technique with o-phenylenediamine substrate. Hybrid cells from selected microcultures were cloned by limiting dilution (100 hybrids per plate). Additional anti-TCA-I antibodies were generated by immunization with Jurkat cells using a similar schedule. Antibody WC12.2, a murine, monoclonal IgG3, anti-TCA-I antibody, was used for the immunochromatological studies due to its ability to bind to Staphylococcal protein A. High-titered antibody was produced in ascites fluid of Pristane (Aldrich, Milwaukee, WI) primed BALB/c mice inoculated intraperitoneally with 1.0 x 10^6 cloned hybridoma cells.

**Antibody purification, conjugation, and isotyping.** Antibody WC12.2 was purified by column immunoadsorption on protein A Sepharose CL-4B (Pharmacia, Piscataway, NJ), eluted with 0.1 mol/L citrate pH 4.0, and neutralized with 0.6 mol/L phosphate pH 8.0. Protein concentration was determined by Coomassie dye binding (BioRad, Richmond, CA). The antibodies produced were isotyped by enzyme-linked immunosorbent assays (ELISAs) using a kit (Zymed, South San Francisco, CA) with isotype-specific rabbit anti-mouse antibodies and a peroxidase conjugated anti-rabbit antibody. In addition, protein A binding ability of the mouse MoAbs was determined by ELISAs using peroxidase conjugated protein A (Zymed). WC12.2 was purified by column immunoadsorption on protein A-Sepharose CL4B (Pharmacia). Purification of WC1.1, a murine monoclonal IgM antibody, was achieved by column chromatography on Sephacryl-300. Fluorescein conjugation was accomplished by applying purified antibody to a Sephadex G25 column equilibrated with 0.15 mol/L phosphate pH 9 to exchange buffers, and then reacted with fluorescein isothiocyanate (Molecular Probes, Eugene, OR). 50 μg in dimethyl sulfoxide (DMSO) per milligram of IgG for 60 minutes at 20°C. Conjugated antibody was isolated by Sephadex G25 column chromatography, which retarded uncoupled fluorescein.

**Surface immunofluorescent staining.** Cells, 0.5 x 10^6, were incubated for 30 minutes at 4°C with saturating amounts of MoAb (usually 10 to 20 μg per 10^6 cells); washed three times with RPMI 1640 containing 5% FCS, 3 mmol/L EDTA, exposed for 30 minutes to a fluorescein-conjugated, goat anti-mouse IgG antibody (Tago, Burlingame, CA); washed; and then fixed in 1% paraformaldehyde. Samples were analyzed using a fluorescence-activated cell sorter (FACS 440, Becton-Dickinson, Mountain View, CA), or a fluorescence microscope.

**Cytoplasmic immunofluorescent staining.** Cells were washed four times in phosphate-buffered saline (PBS) and fixed for 30 minutes at 4°C in PBS containing 1% formaldehyde and 0.05% Nonidet P-40 (NP-40) (Sigma). Cells were then washed twice in PBS, and blocked for 30 minutes with 10% pooled human serum in PBS (10% PBS-PBS), continuing a murine MoAb of irrelevant specificity (antibody 9E8) to reduce background staining. A saturating amount of fluorescein-conjugated MoAb WC12.2 in 10% PBS-PBS was added for 30 minutes, and cells were then washed three times and fixed with 1% paraformaldehyde. Analysis of total cell fluorescence was measured on a FACS 440 with logarithmic amplification.

**Radioimmunoprecipitation.** Jurkat cells (5 x 10^6) were surface-labeled with 125I by the lactoperoxidase technique. Immunoprecipitations were accomplished with 5 μL of WC12.2 ascites, and complexes were collected with 20 μL of 50% (vol/vol) protein A Sepharose CL-4B beads (Pharmacia). One-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in a conventional fashion (described below). Cells activated for 8 days with PHA (purified PHA, Burroughs-Wellcome) were labeled with 35S-methionine by incubating 2 x 10^6 cells in methionine-free RPMI 1640, containing 10% dialyzed FCS and 1 mCi of 35S-methionine for 3 hours. Immunoprecipitation and electrophoresis were performed as outlined above, except that 35S gels were processed with Enhance (New England Nuclear, Boston, MA) for fluorographic development using Kodak X-Omat AR film (Eastman Kodak, Rochester, NY).

**SDS gel electrophoresis.** The SDS-PAGE system of Laemmli as described by Goding was used to separate proteins by molecular radius. The immunoprecipitate sample was boiled in sample buffer (.062 mol/L Tris, pH 6.8, 2% SDS, 10% glycerol, 5% mercaptoethanol) and applied to a 5% stacking gel to concentrate the protein. A 10% running gel was used to resolve TCA-I. Electrophoresis was performed at 20-mA constant current for 4 hours. Gels were then stained with 0.2% Coomassie Blue in 45% methanol, 10% acetic acid in water and destained in 25% methanol, 7.5% acetic acid in water. Gels were then dried on Whatman filter paper with a BioRad gel dryer and autoradiographed.

**Immunoblotting.** Wet SDS-polyacrylamide gels were equilibrated in a blotting buffer (0.02 mol/L Tris,0.15 mol/L glycine, 20% methanol, pH 8). The gel was then sandwiched with nitrocellulose and supporting pads, and inserted in a Transblot apparatus (BioRad) filled with the blotting buffer. The blot was then run at 30 V constant voltage overnight and increased to 60 V for 1 hour the next morning. The sandwich was disassembled and the nitrocellulose paper removed. It was then blocked in Tris-buffered saline (TBS) with 0.1% Tween 20. The nitrocellulose was cut into strips corresponding to the gel lanes. Antigens were visualized using selected primary mouse antibody at 1 to 50 μg/mL applied for 90 minutes. The nitrocellulose was washed three times and developed with the Vector ABC glucose oxidase kit. This included sequential application of biotinylated horse anti-mouse antibody, avidin-biotin-glucose oxidase complex, and nitroblue tetrazolium (NBT) substrate. The intense blue color of the insoluble formazan localized the primary murine MoAb binding.

**Microsequencing of blotted TCA-1.** Detailed structural analysis of amino acid content by microsequencing of N-terminal amino acids was accomplished through the aid of the Protein Chemistry Core Facility at the University of Florida. TCA-1 was isolated from Jurkat cells by immunoprecipitation with WC12.2 crosslinked to protein A-Sepharose beads and subjected to SDS-PAGE. The gel was then electrophoretically transferred to nitrocellulose membranes (Millipore, Bedford, MA) according to the method of Matsudaira, the TCA-1 protein band cut from the membrane and analyzed in an Applied Biosystems 470A Gas Phase Protein Sequencer (Foster City, CA). The TCA-1 protein band was then visualized with a 1:500 dilution of goat anti-mouse Sepharose beads (Pharmacia and Zymed) was then washed three times in 0.05% Tween 20, and then fixed in 1% paraformaldehyde. The gel was then cut into strips corresponding to the gel lanes. Antigens were visualized using selected primary mouse antibody at 1 to 50 μg/mL applied for 90 minutes. The nitrocellulose was washed three times and developed with the Vector ABC glucose oxidase kit. This included sequential application of biotinylated horse anti-mouse antibody, avidin-biotin-glucose oxidase complex, and nitroblue tetrazolium (NBT) substrate.
combined with 5 mg of MoAb diluted in 0.1 mol/L bicarbonate, pH 8.5. After a 30-minute incubation the beads were washed twice in bicarbonate buffer, washed once in 0.2 mol/L triethanolamine, pH 8.2, and reacted with freshly prepared dimethyl pimelimidate 20 mmol/L in 0.2 mol/L triethanolamine, pH 8.2. The reaction proceeded for 45 minutes at room temperature with constant mixing. The reaction was terminated by resuspending the beads in 20 mmol/L ethanolamine in 0.2 mol/L triethanolamine, pH 8.2 for 10 minutes. The beads were then washed twice in PBS and stored at 4°C. Covalently crosslinking the antibody to beads eliminated elution of antibody with TCA-1 before SDS-PAGE, and allowed for clear identification and removal of the 100-Kd TCA-1 fragment after electroblotting to PVDF.

**TCA-1 protein dot immunoblot.** Analysis of total cellular TCA-1 content was achieved by dot immunoblot analysis using a defined standard cell lysate from 5 x 10⁶ Jurkat cells per millilitre of lysate. The cells were pelleted in a conical centrifuge tube, the supernatant discarded, and the cell pellet lysed at 4°C with a buffer containing 0.5 mol/L Tris, 0.15 mol/L NaCl, 5 mmol/L EDTA, 0.5% NP-40 at pH 8.0. Fifty microliters of serial twofold dilutions (starting at 1:10) of the lysate in TBS with 1% bovine serum albumin (BSA) and 0.1% SDS, was applied under suction to the 96-well dot blot apparatus (Schleicher & Schuell, Keene, NH) fitted with 0.2-μm nitrocellulose paper. Staining was maintained for 5 minutes to ensure that all wells were dry. The nitrocellulose was removed and placed in a blocking buffer of TBS, 10% human serum, and 0.05% NP-40 for 1 hour. Monoclonal anti-TCA-1 antibody WC11.3 was added at a final concentration of 10 μg/mL and incubated at 20°C for 30 minutes. The nitrocellulose sheet was then washed twice for 10 minutes each wash with TBS, 1% BSA, and 0.05% NP-40. The dots were then developed with the ABC-glucose oxidase immunodetection kit (Vector), which uses the sequential application of biotinylated horse anti-mouse antibody, avidin-biotin-glucose oxidase complex, and nitroblue tetrazolium (NBT) substrate. A dark blue dot develops wherever the primary WC11.3 antibody binds to TCA-1. The pattern of lectin binding of the TCA-1 glycoprotein was elucidated by incubation of the standard Jurkat lysate, with lectins covalently coupled to Sepharose beads (Vector) before performing the TCA-1 protein dot immunoblot. Twenty-five microliters of standard Jurkat lysate was added to 25 μL of TBS supplemented with 20 mmol/L calcium and magnesium, and incubated with 40 μL of the lectin coupled beads for 90 minutes at 4°C. Beads were collected by a brief centrifugation, and supernatants saved for assay. Beads were washed twice with TBS, 0.05% NP-40, 20 mmol/L calcium/magnesium. Beads were then eluted with specific competing sugars as follows: 0.2 mol/L α-methylmannoside + 0.05% NP-40 for Concanavalin A (ConA) and *Lens culinaris* agglutinin (LCA); 0.3 mol/L glucosamine + 0.1% SDS for wheat germ agglutinin (WGA); and 0.2 mol/L lactose + 0.05% NP-40 for *Ricinus communis* agglutinin I (RCA-I). Eluates were also assayed for TCA-1 by dot immunoblot.

**Tunicamycin treatment.** A stock solution was prepared by dissolving tunicamycin (Sigma) at 1.0 mg/mL in PBS at pH 9.0 (high pH necessary to dissolve the tunicamycin), and filter-sterilized. Dilutions from 1:100 (0.1 μg/mL) to 1:10,000 (0.1 μg/mL) in RPMI 1640 with 12% FCS were made, and selected cell lines were cultured in these media for 48 hours. Cells were then examined for surface membrane TCA-1 by indirect immunofluorescence.

**Immunohistochemistry.** Cryostat tissue sections or cytospin cell preparations were fixed in cold acetone for 10 minutes, washed in PBS for 10 minutes, and blocked with 5% horse serum for 1 hour. Primary mouse antibody diluted in blocking buffer was added at appropriate dilutions, usually 1:200 to 1:500, and incubated for 1 hour. After washing in PBS the slides were sequentially exposed to biotinylated horse anti-mouse antibody, avidin-biotin-glucose oxidase complex, and nitroblue tetrazolium substrate (Vector labs). The slides were counterstained in Nuclear Fast Red, washed, dehydrated, and mounted with Permount (Fisher Scientific, Fair Lawn, NJ).

**RESULTS**

**Distribution of TCA-1.** TCA-1 is found primarily in the cytosol of neoplastic T cells. Expression of TCA-1 is greatest in Jurkat, MT1, HUT102, and HUT78 cell lines, and to a much less extent in PHA-activated T cells and SH cells. Other T-cell lines (MOLT4, HS2B, DND41, 8402, CEM, HPB-ALL, and HPB-MLT), B-cell lines (NALM6, Raji, Daudi, and SHB), nonlymphoid lines (HL60, K562, HEL, U937, and UF-SK1), as well as normal human bone marrow, lymph node and thymic cells, were found not to express TCA-1 (Table 1). An exhaustive survey of other normal human tissues both in the investigator's laboratory and in other laboratories participating in the Third International Workshop and Conference on Human Leukocyte Differentiation Antigens (Oxford, England, September 21-26, 1986) confirmed that no other tissues express TCA-1. Examination of fresh lymphoblasts from patients with T-cell malignancies showed that 2 of 4 leukemias and 1 of 3 lymphomas tested expressed TCA-1. All cells expressing TCA-1 did so predominantly intracellularly in the Golgi area. Additional surface membrane TCA-1 was observed on SH cells and the Jurkat cell line only. Although the antigen expression by SH cells can be augmented by culture in IL-2, the quantity of TCA-1 expressed is much less than in Jurkat cells (Fig 1).

**Subcellular localization of TCA-1.** Examination of Jurkat cells by cytoplasmic immunofluorescent staining and fluorescence microscopy showed that the majority of TCA-1 localized in the perinuclear membrane stacks of the Golgi apparatus (Fig 2). The staining pattern of the anti-TCA-1 antibody WC12.2 is identical to the pattern obtained with the MoAb 53FC3, which specifically binds to the Golgi.

**Anti TCA-1 antibody does not affect cell proliferation.** Although we were unable to detect TCA-1 on the surface of

<table>
<thead>
<tr>
<th>Table 1. Distribution of TCA-1</th>
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<tr>
<td>Cell Lines</td>
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<tr>
<td><strong>T-cell lineage</strong></td>
</tr>
<tr>
<td>Jurkat</td>
</tr>
<tr>
<td>HUT102, MT1, HUT78</td>
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<tr>
<td>SH</td>
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<tr>
<td>MOLT4, DND41, 8402, CEM, HPB-ALL, HPB-MLT</td>
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<tr>
<td><strong>B-cell lineage</strong></td>
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<tr>
<td>NALM6, Raj, Daudi, SHB</td>
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<td>Nonlymphoid lines</td>
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<td>HL60, K562, HEL, U937, UF-SK1</td>
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<tr>
<td><strong>Normal Human Tissues</strong></td>
</tr>
<tr>
<td>PHA blasts (day 6)</td>
</tr>
<tr>
<td>T-cell clone</td>
</tr>
<tr>
<td>PHA blasts (day 3)</td>
</tr>
<tr>
<td>Bone marrow</td>
</tr>
<tr>
<td>Lymph node</td>
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<td>Tonsil</td>
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<td>Spleen</td>
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<td>Thymus</td>
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<td>Skin, muscle, brain, liver, kidney, lung, gut, gonads</td>
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+ +, strongly positive; +, weakly positive; −, negative.
activated T cells and the majority of T-cell neoplasms by conventional techniques, it is possible that anti-TCA-I antibodies might inhibit T-cell growth by interrupting some autocrine mechanism. However, when PHA-activated T cells, SH cells, or Jurkat cells were cultured in the presence of antibody WC12.2, no difference in cell proliferation was observed when compared with control cultures.

**Biochemistry.** SDS-PAGE analysis of TCA-I immunoprecipitated from ^125^I surface-labeled Jurkat cells produced a single band with a molecular weight (mol wt) of 120 Kd (Fig 3A). This result was the same under reducing and nonreducing conditions. No change was observed after harsh treatment of the immunoprecipitate with 50 mmol/L triethylamine pH 11, 0.2 mol/L citrate pH 4, or 1.0 mol/L NaCl, all of which suggests a monomeric structure for TCA-I. Sequential immunoprecipitation and separate cocapping and modulation experiments have shown no relationship between TCA-I and the 120-Kd molecule recognized by CD6 antibodies. Polyclonal antibodies to the human immunodeficiency virus 1 (HIV-1) gp120 protein also fail to block anti-TCA-I antibody binding. To confirm the identity between surface and intracellular TCA-I, similar studies were performed using PHA-activated T cells (day 8), metabolically labeled with ^35^S-methionine. A polypeptide with a mol wt of 120 Kd was again immunoprecipitated (Fig 3B), although this band may actually be a doublet indicating minor glycosylation differences between cytoplasmic and surface TCA-I. Further analysis of this phenomenon is limited by the resolution of the SDS-PAGE technique. Experiments using ^2^P orthophosphate-labeled PHA blasts demonstrated that TCA-I is not phosphorylated when compared with a positive control murine monoclonal anti-Sm antibody (data not shown).

Other immunoprecipitation experiments with tunicamycin-treated Jurkat cells have shown that the mol wt of TCA-I is reduced by 5 Kd when compared with untreated cells (Fig 4). This indicates that the molecule is a glycoprotein with N-linked carbohydrate. The carbohydrate is not involved in the antibody binding site since the antigen still immunoprecipitated with WC12.2.

In addition, the distribution and intensity of TCA-I expression in several cell lines were not affected by culture in the presence of tunicamycin, an inhibitor of N-linked glycosylation (Table 2). This eliminates the possibility that N-linked carbohydrate is either part of the epitopes recognized by the four anti-TCA-I antibodies WC1.1, WC12.2, WC13.1, and WC11.3 (in TCA-I positive cells), or masks the epitopes (in TCA-I negative cells).

Further data defining the carbohydrate structure of TCA-I was derived from experiments with immobilized lectins.

**Fig 1.** Cell fluorescence histograms produced by FACS analysis of the indicated cell types after surface (left) or surface plus intracellular (total cell, right) staining with antibody WC12.2 (---) and negative control antibody 9E8 (----).
NEW T-LINEAGE GLYCOPROTEIN TCA-1

Table 2. Surface Membrane TCA-1 Expression After 48 Hours of Culture in Tunicamycin

<table>
<thead>
<tr>
<th>Tunicamycin Concentration (µg/mL)</th>
<th>Jurkat</th>
<th>HUT78</th>
<th>MOLT4</th>
<th>NALM6</th>
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<tr>
<td>0</td>
<td>+</td>
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<tr>
<td>0.1</td>
<td>+</td>
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<tr>
<td>1.0</td>
<td>+ *</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>10.0</td>
<td>+ *</td>
<td>-</td>
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*Diminished cell viability seen at this concentration.

Figure 3. SDS-PAGE analysis of radioimmunoprecipitates of surface 35S-labeled Jurkat cells (A) and 35S methionine metabolically labeled, day 6, PHA blasts (B). WC12.2 recognizes TCA-1, and antibodies 35.1 (CD2), WC11.5 (CD6), and 10.2 (CD6) are included as controls. Lane WC12.2 (NR) was from a nonreduced sample, while all other lanes were run under reducing conditions.

Figure 5 illustrates the selective binding of the TCA-1 glycoprotein to the lectin Ricinus communis-1 (castor bean) agglutinin (RCA-1) and specific elution with the competing sugar, lactose. Some degree of nonspecific binding of TCA-1 to the various bead preparations was observed as reduced amounts of TCA-1 in many of the supernatant lanes. However, specific elution with competing sugars indicated that only RCA-I lectin specifically bound appreciable amounts of TCA-1 glycoprotein. From these studies we conclude that terminal D-galactose (or possibly N-acetylgalactosamine) units are present in the oligosaccharide portion of the TCA-1 glycoprotein. ConA and LCA, which recognize α-linked mannose, and WGA, which recognizes N-acetylgalactosamine, failed to bind appreciable amounts of TCA-1.

Microsequencing of TCA-1. A detailed knowledge of the molecular structure of TCA-1 may provide additional clues as to its function in neoplastic T cells. Detailed structural analysis of amino acid content and microsequencing of N-terminal amino acids has been accomplished by analysis of native TCA-1 from Jurkat cells electroblotted to PVDF membranes, according to the method of Matsudaira.

DISCUSSION

TCA-1 expression occurs only in cells of the T-lymphocyte lineage. Certain neoplasms of mature T cells contain the largest quantities of TCA-1 glycoprotein yet observed. Studies of its cell distribution and biochemistry distinguish TCA-1 from previously described T-cell proteins and activation-related molecules. Other proteins that are unique to T lymphocytes have proven to play a vital role in the biology of T-cell function. The Ti/CD3 antigens, for example, were initially described as antigens unique to T cells, and were later shown to be the T-cell receptor for antigen and its associated signal transduction proteins.

Because of its restriction to T cells, it seems likely that TCA-1 plays some unique role in the intracellular environment of activated or transformed T cells. This glycoprotein is not simply associated with T-cell proliferation, since some rapidly proliferating T-cell leukemias and PHA-activated normal T cells clearly lack TCA-1. It is possible that this glycoprotein is the translation product of a T-cell-specific oncogene or is in some way responsible for the malignant transformation of certain T lymphocytes.

TCA-1 is an intracellular glycoprotein that is detected primarily in the Golgi stacks. This organelle is responsible for...
remodeling N-linked oligosaccharides attached to proteins through the phospholipid dolichol in the endoplasmic reticulum. Glycoproteins processed in the Golgi have one of three subsequent destinations: secretory vesicles, the plasma membrane, or lysosomes. Lysosomes are cytosolic organelles whose components do not reach the cell surface membrane, so the observed expression of TCA-1 on the plasma membrane in Jurkat and SH cells is incompatible with any hypothesis that TCA-1 is a lysosomal component. The TCA-1 glycoprotein appears on the surface membrane of Jurkat and SH cells, but other cells that exuberantly express intracellular TCA-1 lack it on the cell surface membrane. It is possible that a protein cleavage step or other post translational modification of TCA-1 occurring along the journey between the Golgi and the cell surface membrane changes its antigenicity in the majority of cells, but not in Jurkat and SH cells. An alternative explanation for the cellular role of TCA-1 is that it may be a secreted glycoprotein that has some autocrine function in certain cells with plasma membrane receptors for TCA-1. In this circumstance one would expect to detect surface membrane TCA-1 as the ligand binds to its receptor. Evidence against this hypothesis comes from 35S-methionine pulse-chase experiments that demonstrate labeled TCA-1 on the surface membrane, but none secreted into the culture supernatant. We have also not been able to dislodge surface membrane TCA-1 by treatment of cells with chaotropic salts, suggesting that it may be an integral membrane glycoprotein and not a loosely bound ligand. In summary from the available experimental data, it seems most likely that TCA-1 is an integral plasma membrane glycoprotein and not secreted from the cell or incorporated into lysosomes.

Intriguing structural data obtained from the partial amino acid sequence of TCA-1 shows homology with a human Ig heavy chain pseudogene. Many important regulatory molecules of T cells and other lymphohematopoietic cells belong to the Ig gene superfamily. These proteins are multi-domain structures, sharing considerable homology in these recurring sequences resembling Ig variable and constant domains. The superfamily includes the T-cell receptor $\alpha$ and $\beta$ chains, the CD3 $\gamma$, $\delta$, and $\epsilon$ chains, CD1, CD2, CD4, CD7, CD8, CD28, major histo compatibility complex class I and II molecules, and $F_c$ receptors. All of these molecules play important roles in T-cell recognition and activation. Only through molecular cloning and complete sequence analysis of TCA-1 will we have sufficient data to determine the degree of homology with other members of the superfamily. Further study of TCA-1 may provide insight into the events that transform normal T cells into malignant ones. Ways may be found to alter the transformed or activated T lymphoblast to promote selective inhibition of these deleterious cells. TCA-1 may play a crucial role in maintaining the transformed or activated state, thus providing an ideal molecular target for the development of new antineoplastic drugs for T-cell leukemias and lymphomas. We plan to test these hypotheses about the role of TCA-1 in T-cell neoplasia by isolating and sequencing the gene through molecular cloning. and subsequently examining the effects of deliberately eliminating or enhancing TCA-1 expression in malignant and normal T lymphocytes.

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Characterization of a new T-lineage glycoprotein expressed in mature T-cell leukemias and lymphomas

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