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TAPA-1, the Target of an Antiproliferative Antibody, Defines a New Family of Transmembrane Proteins

RACHEL OREN,† SHUJI TAKAHASHI, CAROL DOSS, RONALD LEVY, AND SHOSHANA LEVY*
Stanford University School of Medicine, Stanford, California 94305

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A murine monoclonal antibody was identified by its ability to induce a reversible antiproliferative effect on a human lymphoma cell line. Immunoprecipitation studies revealed that the antibody reacted with a 26-kilodalton cell surface protein (TAPA-1). A diverse group of human cell lines, including hematolymphoid, neuroectodermal, and mesenchymal cells, expressed the TAPA-1 protein. Many of the lymphoid cell lines, in particular those derived from large cell lymphomas, were susceptible to the antiproliferative effects of the antibody. TAPA-1 may therefore play an important role in the regulation of lymphoma cell growth. A cDNA clone coding for TAPA-1 was isolated by using the monoclonal antibody to screen an expression library in COS cells. Analysis of the deduced amino acid sequence indicated that the protein is highly hydrophobic and that it contains four putative transmembrane domains and a potential N-myristoylation site. TAPA-1 showed strong homology with the CD37 leukocyte antigen and with the ME491 melanoma-associated antigen, both of which have been implicated in the regulation of cell growth.

The interaction of a cell with its environment is dependent on structures anchored in the membrane that act as receptors and transmitters of a variety of signals for the regulation of gene expression and for growth. Many receptors have been identified by using purified hormones or growth factors as binding ligands. However, it is likely that a large number of molecules that are important in the regulation of cell growth have yet to be identified because their ligands are unknown. We have developed an approach to identify such cell surface molecules by screening for antibodies that disrupt cell growth (30). We reasoned that antibodies that recognize cell surface structures important for growth regulation might interfere with these functions. Such antibodies, rather than specific ligands, could then be used to isolate and characterize novel receptors or signal transducers. Here we report the isolation of one such antibody, which binds a 26-kilodalton (kDa) cell surface protein that we have called TAPA-1 (the target of an antiproliferative antibody). This antibody reacts with most but not all human cell lines tested and has a marked antiproliferative effect on some of them. This antibody was used to isolate a cDNA clone from a mammalian expression library (21). The deduced amino acid sequence of TAPA-1 shows strong homology to the CD37 antigen, which is highly expressed on B lymphocytes (6), and to ME491, which is expressed during the early stages of melanoma and is lost during malignant progression (10).

MATERIALS AND METHODS

Cell lines and activated peripheral blood lymphocytes. OCI-LY8 was obtained from M. Tweeddale and H. Messner, the Ontario Cancer Institute (29). JP-DLCL-1 was initiated in our laboratory by A. Zelenetz (unpublished data). RS 11846 was obtained from R. Morgan and F. Hecht, Southwest Biomedical Research Institute, Scottsdale, Ariz. SUP-(B8, SUP-B17, SUP-B12, and SUP-T13) (31) were isolated by S. Smith, Children's Hospital, Stanford. All other cell lines are available from the American Type Culture Collection, Rockville, Md.

Peripheral blood lymphocyte proliferation. Mononuclear cells were separated from fresh heparinized blood of a normal volunteer by Ficoll-Hypaque density gradient centrifugation. Cells were suspended in RPMI 1640 supplemented with 2% heat-inactivated fetal bovine serum, glutamine (2 mM), penicillin (50 μg/ml), and streptomycin (50 μg/ml); 5×10^4 cells in 100 μl were seeded in triplicate microwell cultures. After 72 h, [^{3}H]thymidine (1 μCi per well; Amersham Corp., Arlington Heights, Ill.) was added, and incubation was continued for 10 to 16 h. [^{3}H]thymidine uptake was measured in a beta scintillation counter (model LS9000; Beckman Instruments, Inc., Fullerton, Calif.).

Production and screening of antiproliferative antibodies. Hybridomas were produced from mice that were injected with OCI-LY8 cells essentially as previously described (30). A proliferative assay with 3(4,5)-dimethylthiazol-2,5-diphenyl tetrazolium bromide (Sigma Chemical Co., St. Louis, Mo.) has been described (30). Briefly, 2×10^3 OCI-LY8 cells suspended in culture medium containing monoclonal antibody were incubated in 96-well flat-bottom plates at 37°C. At the end of 3 days, proliferation was estimated as follows: 3(4,5)-dimethylthiazol-2,5-diphenyl tetrazolium bromide, which produces an insoluble purple formazan product in the presence of mitochondrial enzymes in living cells, was added. The crystals were solubilized by adding 150 μl of isopropanol in 0.04 N HCl. The absorbance of each well was quantified by a multiwell scanning photometer (MicroELISA MR580; Dynatech Laboratories, Inc., Alexandria, Va.) by using a test wavelength of 570 nm and a reference wavelength of 630 nm.

Immunoprecipitation. Cells were surface labeled with ^{125}I by using the lactoperoxidase-glucose oxidase method or biosynthetically labeled by incubation in [^{35}S]methionine as previously described (15). They were then solubilized in 1.0% Triton X-100 buffer (150 mM NaCl, 10 mM Tris, 0.02% NaN_3, 1 mM phenylmethylsulfonyl fluoride, 1.0 μg of pepstatin A per ml, 0.1 U of aprotinin per ml, and 10 mM iodoacetamide; all from Sigma). Samples of 100 μl of lysate were added to 30 μl of goat anti-mouse immunoglobulin G

* Corresponding author.
† Present address: Department of Biophysics, Weizmann Institute of Science, Rehovot, Israel.

(TAGO, Burlingame, Calif.). Sepharose 4B beads (Pharmacia Fine Chemicals, Piscataway, N.J.) preconjugated with 3 μg of specific antibody were incubated overnight at 4°C and then washed four times with phosphate-buffered saline (PBS) containing 0.02% NaN_3, 3 mg of methionine per ml, 0.5% Nonidet P-40 (BDH, Poole, England), 0.025% sodium dodecyl sulfate (SDS), 0.5% deoxycholate, and 10 mM iodoacetamide (all from Sigma). Nonreduced samples were boiled in sample buffer (62.5 mM Tris, 3% SDS, 10% Glycerol) and analyzed by SDS-polyacrylamide gel electrophoresis (13). Reduced samples were handled identically, except that 5% 2-mercaptoethanol was added to the sample buffer. Radiolabeled proteins were visualized by autoradiography with Kodak XAR-5 film and an intensifying screen.

RNA preparation and poly(A)$^+$ RNA selection. Total RNA from OCI-LY8 cells was prepared by washing the cells with PBS, followed by lysis with 5 M guanidinum thiocyanate–10 mM EDTA–10 mM Tris (pH 7.5)–0.7 M 2-mercaptoethanol. The lysate was adjusted to contain 1.9 M CsCl, 40 mM EDTA, and 0.8% Sarkosyl. The mixture was then layered over a cushion of 5.7 M CsCl–10 mM EDTA and centrifuged at 36,000 rpm for 20 h at 20°C in a Beckman SW41 rotor (5). Poly(A)$^+$ RNA was prepared by oligo(dT)-cellulose chromatography (1).

Preparation of the cDNA library. Double-stranded cDNA was made from 3 μg of poly(A)$^+$ RNA by the protocol of Gubler and Hoffman (8) with a cDNA synthesis system (Amersham). The cDNA was ligated to kinase-treated *Bst*xI linkers: a combination of an 8-mer (5′ CTGGAAAG) and a 12-mer (5′ CTTTCCAGCACA) (21). Removal of excess linkers and size selection of cDNA were performed by gel electrophoresis on 1% agarose. DNA longer than 700 base pairs was excised and eluted from the gel by glass powder (Geneclean; Bio 101, La Jolla, Calif.). The double-stranded cDNA was then ligated into the pCDM8 expression vector (21), which had been cut with *Bst*xI and gel purified as described above. The ligated material was electroporated into *Escherichia coli* MC1061 p3; the resulting cDNA library contained 2×10^5 independent clones.

Transfection of the cDNA into COS cells. (i) Transfection by DEAE dextran. DEAE dextran (Pharmacia) was used to introduce the cDNA library into COS cells. Cells in 10 cm dishes at 50% confluence were transfected in 4 ml of Dulbecco modified Eagle medium (DMEM) containing 10% Nu Serum (Collaborative Research, Inc., Waltham, Mass.), 400 μg of DEAE dextran per ml, 100 μM chloroquine diphosphate, and CsCl-purified DNA at 0.5 to 5 μg/ml. After 3.5 h at 37°C, the transfection mixture was removed, and the cells were treated with 10% dimethyl sulfoxide in PBS for 2 min. Cells were then returned to DMEM–10% fetal calf serum. After incubation for 24 h, the cells were removed by trypsinization, washed to remove residual DEAE dextran, and replated to allow cell surface protein expression for another 24 to 72 h. Three rounds of DEAE dextran transfection were followed by a round of spheroplast fusion.

(ii) Transfection by spheroplast fusion. *E. coli* MC1061 p3 cells containing plasmids were grown to an optical density at 600 nm of 0.4. Chloramphenicol (150 μg/ml) was then added, and the incubation was continued at 37°C for 10 to 14 h. The bacteria were converted into spheroplasts by incubation with lysozyme. The spheroplast suspension was layered over 50% confluent COS cells in a 10-cm dish by centrifugation at 1,000 × g for 10 min, and 5 ml of 50% (vol/vol) polyethylene glycol 1500 in DMEM was added to the center of the dish. After 120 to 150 s of contact the polyethylene glycol was washed off with DMEM, and the COS cells were allowed to grow in DMEM–10% fetal calf serum containing 15 μg of gentamicin sulfate per ml (21).

Panning of transfected COS cells. Transfected COS cells that expressed specific cell surface antigens were isolated by panning (21). Goat anti-mouse immunoglobulin G (affinity purified) was used to coat 60-mm Falcon bacteriological plates. Transfected COS cells were detached from the tissue culture dish in 5 mM EDTA in PBS, washed, and reacted with the relevant mouse monoclonal antibody for 0.5 to 1 h on ice. The cells were washed three times with 5 mM EDTA–PBS–5% fetal calf serum–0.02% NaN_3, suspended in the same buffer, and placed on the goat anti-mouse immunoglobulin G-coated plates for 1 to 3 h at room temperature. Nonadherent cells were removed from the plate by gentle washing with PBS–5% fetal calf serum. Plasmid DNA was recovered from adhered cells by the Hirt procedure (9). Then 0.8 ml of 10 mM EDTA–0.6% SDS was added per plate, and plates were incubated for 20 min. The lysates were collected, and high-molecular-weight DNA was precipitated with 0.2 ml of 5 M NaCl, overnight incubation at 4°C, and then centrifuged for 4 min at room temperature. The low-molecular-weight supernatant DNA was purified by phenol extraction and used for electroporation of *E. coli* MC1061 p3.

DNA and protein sequence analysis. Sequencing of the cDNA clone was performed by subcloning fragments into M13mp18 and M13mp19 (17). Single-stranded DNA was obtained and used for dideoxy sequencing (20) with a Sequenase kit (U.S. Biochemical Corp., Cleveland, Ohio) and [^{35}S]dATP. The sequences were read completely from both strands by using M13 universal primer and internal primers. GenBank, SWISS-PROT, and NBRF/PIR data bases were searched with the FASTN and FASTP programs (19). Analysis of hydropathicity by the method of Kyte and Doolittle (12), and analysis of amphipathicity was by the method of Margalit et al. (16). The TULLA program was used for sequence alignments (23).

RESULTS

Isolation of antiproliferative monoclonal antibodies. Mice were immunized with tumor cells taken from patients with lymphoma or with cell lines derived from such patients. Hybridomas made from immunized mice were screened for their ability to inhibit the proliferation of the human lymphoma cell line OCI-LY8. Thirty-five independent hybridomas that produced antibodies with antiproliferative effects were identified (Table 1). The molecules recognized by these antiproliferative antibodies were characterized by immunoprecipitation of radioiodinated cell surface proteins from the OCI-LY8 cells. Many of the antiproliferative antibodies reacted with previously known molecules such as MHC class I or class II, immunoglobulin, or the transferrin receptor. A number of the antiproliferative antibodies failed to identify a cell surface protein by this method of analysis. Several antiproliferative antibodies reacted with cell surface protein molecules that have not been previously characterized, including a 110-kDa molecule that was identified by five independent hybridomas, a 28-kDa molecule, and a 26-kDa molecule. This latter 26-kDa protein (TAPA-1), recognized by monoclonal antibody 5A6 (immunoglobulin γ1), was selected for detailed analysis. TAPA-1 showed an identical apparent molecular weight under either reducing or nonreducing conditions and could be labeled equally well by cell surface radioiodination or by biosynthetic labeling with [^{35}S]methionine.

TABLE 1. Hybridomas producing antiproliferative antibodies

Source of immunizing cells	Type of malignancy[a]	No. of anti-proliferative MAb[b]	Known antigen (no.)[c]	Size (kDa) of unknown antigen (no.)[c]
B cell from patient 1	FL	2	Transferrin receptor (1)	
B cell from patient 2	DLCL	5	Immunoglobulin (2)	55 (1)
B cell (OCI-LY8)	DLCL	3	Immunoglobulin (1)	26 (1)
B cell (SU-DHL4)	DLCL	9	MHC[d] class II (3)	
T cell from patient 3	MF	3		110 (2)
T cell from patient 4	MF	2		28 (1)
T cell from patient 5	CLL	1		
T cell from patient 6	CLL	6	MHC class I (3)	110 (2)
T cell from patient 7	MF	1	MHC class II (1)	
T cell (SUP-T13)	ALL	3	MHC class I (1)	110 (1)

[a] DLCL, Diffuse large cell lymphoma; FL, follicular lymphoma; MF, mycosis fungoides; CLL, chronic lymphocytic leukemia; ALL, acute lymphocytic leukemia.
[b] MAb, Monoclonal antibodies.
[c] Determined by immunoprecipitation of radioiodinated cell surface proteins followed by SDS-polyacrylamide gel electrophoresis.
[d] MHC, Major histocompatibility complex.

Antibodies against TAPA-1 induce a potent and reversible antiproliferative effect. In the initial screening assay for antiproliferative antibodies, the target cells were incubated with hybridoma supernatants for 72 h before they were assayed for growth inhibition. An experiment was designed to determine the potency, time course, and reversibility of the antiproliferative effect of antibody 5A6 (Fig. 1). A series of dilutions of this antibody were mixed with OCI-LY8 cells and either left in the culture continuously for the entire 72-h period or removed by dilution of the culture medium sixfold with fresh medium. The 5A6 antibody had potent antiproliferative effects on these cells at concentrations as low as 3 ng/ml. However, this effect was reversible by removal of the monoclonal antibody as late as 19 h after the initial exposure. After that point, cell growth was irreversibly inhibited.

Expression of TAPA-1 by human cell lines. A variety of lymphoid and nonlymphoid human cell lines were analyzed for reactivity with the 5A6 antibody. Most of the human cell lines tested, including those of lymphoid, myeloid, and mesenchymal origin, were reactive (Table 2). The single exception to this was the U937 histiocytic lymphoma cell line. Cell lines of mouse and monkey fibroblast origin did not react with the 5A6 antibody. Analysis of normal peripheral blood cells showed that the 5A6 antibody reacted with lymphocytes but only very weakly with monocytes or granulocytes. These results, obtained by cytofluorometry, were confirmed by immunoprecipitation of cell surface and biosynthetically labeled proteins from their respective cell lines, which confirmed the presence of the 26-kDa protein in several cell lines that reacted with the 5A6 antibody (Fig. 2a). Although the expression of TAPA-1 was rather ubiquitous among human cell lines, the sensitivity of these cells to

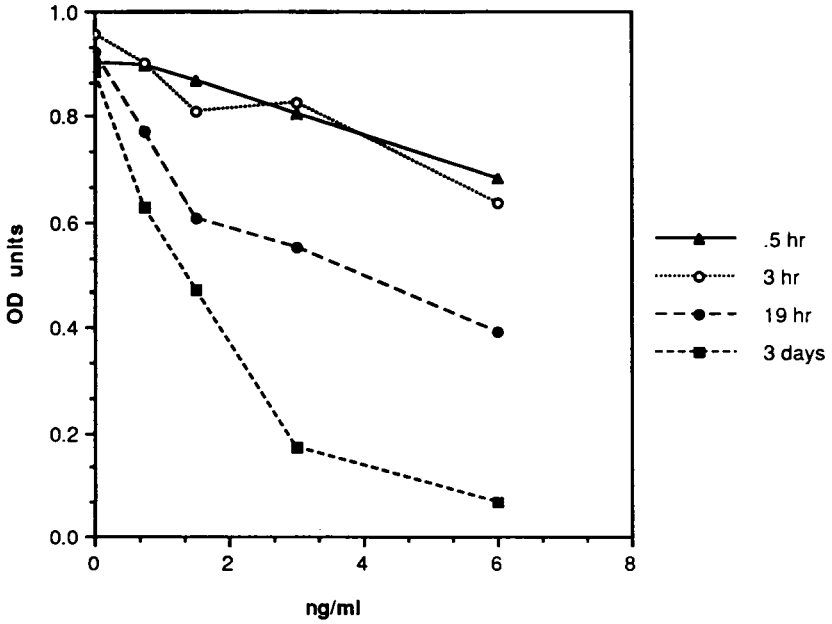

FIG. 1. Reversibility of the 5A6 antibody effect. OCI-LY8 cells were incubated with increasing antibody concentrations continuously for 3 days, or in parallel cultures the antibodies were diluted sixfold with medium (0.5, 3, or 19 h after the initiation of the culture). After 3 days, cell proliferation was determined by the 3(4,5)-dimethylthiazol-2,5-diphenyl tetrazolium bromide assay. The ordinate represents the mean optical density (OD) units of triplicate wells. The antibody was cytotoxic when present at 3 ng/ml, but its effect was reversible within the first 19 h.

TABLE 2. Antiproliferative effects on cell lines

Cell line	Origin[a]	Surface expression[b]	Growth inhibition (%)[c]
OCI-LY8	DLCL	++	100
SU-DHL-4	DLCL	++	100
JP-DLCL-1	DLCL	++	65
RS11846	DLCL	±	3
SUP-B8	Burkitt's lymphoma	++	54
SUP-B17	Burkitt's lymphoma	++	12
SUP-B12	Burkitt's lymphoma	++	7
Daudi	Burkitt's lymphoma	+	7
Raji	Burkitt's lymphoma	+	0
Reh	pre-B-cell ALL	++	0
Nalm-1	pre-B-cell ALL	++	0
SB	EBV B cell	+	60
UC729-6	EBV B cell	+	34
8866	EBV B cell	+	1
SUP-T13	T-cell ALL	++	29
Jurkat	T-cell ALL	++	12
HPB-ALL	T-cell ALL	++	0
Peer	T-cell ALL	++	0
Molt-4	T-cell ALL	+	2
Resting lymphocytes[d]	Normal blood	+	ND[e]
Activated by PHA		ND	31
Activated by OKT3		ND	48
Monocytes	Normal blood	±	ND
Granulocytes	Normal blood	±	ND
KG-1	Acute myelogenous leukemia	+	14
U937	Hystiocytic lymphoma	−	0
K562	Chronic myelogenous leukemia	++	0
HL60	Promeyelocytic leukemia	±	0
T24	Bladder carcinoma	±	0
SK-NSH	Neuroblastoma	++	0
SK-MEL28	Melanoma	+	0
3T6	Mouse fibroblasts	−	0
COS7	Monkey fibroblast	−	0

[a] DLCL, Diffuse large cell lymphoma; ALL, acute lymphocytic leukemia; EBV, Epstein-Barr virus.

[b] Determined by flow cytometry. Mean channel fluorescence intensity compared with negative control staining according to the following scale: −, 0; 1 to 5, ±; 5 to 20, +; >20, ++.

[c] Determined by 3(4,5)-dimethylthiazol-2,5-diphenyl tetrazolium bromide assay in the presence of 5A6 (10 μg/ml).

[d] Peripheral blood lymphocytes were stimulated with phytohemagglutinin (5 μg/ml) or OKT3 (hybridoma supernatant diluted 1:6). Percent growth inhibition was calculated as follows: 1 − [mitogen stimulated (cpm) − mitogen stimulated with 5A6 (cpm)]/[mitogen stimulation (cpm) − unstimulated (cpm)] × 100, where cpm is counts per minute.

[e] ND, Not determined.

the antiproliferative effects of the 5A6 antibody was selective. The cell lines that were growth inhibited the most included OCI-LY8, SU-DHL4, and JP-DLCL-1, all derived from B-cell lymphomas of the diffuse large cell type. Some cell lines such as Raji and HPB-ALL, which expressed TAPA-1 in equivalent amounts, were unaffected by the 5A6 antibody. The molecular weight of the TAPA-1 molecule

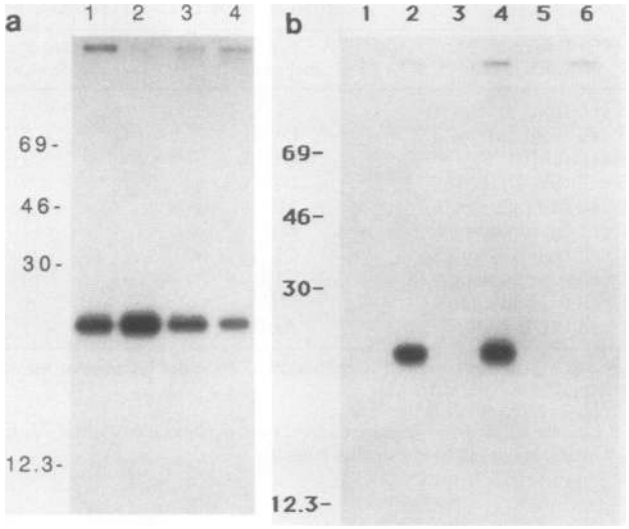

FIG. 2. (a) Immunoprecipitation of TAPA-1. 5A6-sensitive OCI-LY8 (lane 1) and SUP-T13 (lane 3) cells and 5A6-insensitive Raji (lane 2) and HPB-ALL (lane 4) cells were radioiodinated, lysed in detergent, and precipitated with the 5A6 antibody. The class-matched control showed no precipitable band in any of these cell lines (data not shown). The immunoprecipitated material was solubilized, subjected to SDS–12.5% polyacrylamide gel electrophoresis, and exposed to X-ray film. (b) Expression of the native and transfected *tapa-1* gene. OCI-LY8 cells (lanes 1 and 2) or COS cells (lanes 3 through 6) transfected with pCDM8*tapa*-1 (lanes 3 and 4) or pCDM8 (lanes 5 and 6) were radioiodinated, lysed in detergent, and precipitated with the 5A6 antibody (lanes 2, 4, and 6) or a class-matched control antibody (lanes 1, 3, and 5).

expressed by all cell lines was the same regardless of their sensitivity to the antiproliferative effects of the 5A6 antibody (Fig. 2a). As expected, the U937 cell line, which expressed no detectable TAPA-1, was unaffected by the 5A6 antibody. Thus, it appeared that the expression of TAPA-1 was necessary but not sufficient to render the cell susceptible to the antiproliferative effects of the 5A6 monoclonal antibody.

Cloning of the *tapa-1* gene. A cDNA library was made from the OCI-LY8 cell line and inserted into the pCDM8 mammalian-bacterial shuttle vector described by Seed and Aruffo (21). By using the approach described by those authors, the library was transfected into COS cells, which were then reacted with the 5A6 monoclonal antibody and panned onto plastic dishes coated with anti-mouse immunoglobulin antibodies. Low-molecular-weight DNA was extracted from cells adherent to the dishes and electroporated into bacteria. By repeated shuttling and enrichment of the library between mammalian cells and bacteria, three independent cDNA clones were isolated that contained an identical 1.5-kilobase (kb) insert. One of these clones (pCDM8*tapa*-1) was retransfected into COS cells, and after 48 h cell surface proteins were radioiodinated and immunoprecipitated with the 5A6 antibody (Fig. 2b). The transfected COS cells expressed an immunoprecipitable molecule identical in size to that expressed by the original OCI-LY8 cell. This identity was also demonstrated by biosynthetic labeling of the cells with [^{35}S]methionine. By neither labeling method was any immunoprecipitable 26-kDa protein detected in COS cells transfected by the pCDM8 vector alone.

Nucleic acid sequence analysis. The nucleic acid sequence of the 1.5-kilobase cDNA insert is shown in Fig. 3. There is

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CCATTGTGCTGGAAAGGCGCGCAACGGCGGCGACGGCGGCGACCCCACCGCGCATCCTGC   60
CAGGCCTCCGCGCCCAGCCGCCCACGCGCCCCCGCGCCCCGCGCCCCGACCCTTTCTTCG  120
CGCCCCCGCCCCTCGGCCCGCCAGGCCCCCTTGCCGGCCACCCGCCAGGCCCCGCGCCGG  180
CCCGCCCGCCGCCCAGGACCGGCCCGCGCCCCGCAGGCCGCCCGCCGCCCGCGCCGCCAT  240
                                                          M     1

GGGAGTGGAGGGCTGCACCAAGTGCATCAAGTACCTGCTCTTCGTCTTCAATTTCGTCTT  300
 G  V  E  G  C  T  K  C  I  K  Y  L  L  F  V  F  N  F  V  F    21

CTGGCTGGCTGGAGGCGTGATCCTGGGTGTGGCCCTGTGGCTCCGCCATGACCCGCAGAC  360
 W  L  A  G  G  V  I  L  G  V  A  L  W  L  R  H  D  P  Q  T    41

CACCAACCTCCTGTATCTGGAGCTGGGAGACAAGCCCGCGCCCAACACCTTCTATGTAGG  420
 T  N  L  L  Y  L  E  L  G  D  K  P  A  P  N  T  F  Y  V  G    61

CATCTACATCCTCATCGCTGTGGGCGCTGTCATGATGTTCGTTGGCTTCCTGGGCTGCTA  480
 I  Y  I  L  I  A  V  G  A  V  M  M  F  V  G  F  L  G  C  Y    81

CGGGGCCATCCAGGAATCCCAGTGCCTGCTGGGGACGTTCTTCACCTGCCTGGTCATCCT  540
 G  A  I  Q  E  S  Q  C  L  L  G  T  F  F  T  C  L  V  I  L   101

GTTTGCCTGTGAGGTGGCCGCCGGCATCTGGGGCTTTGTCAACAAGGACCAGATCGCCAA  600
 F  A  C  E  V  A  A  G  I  W  G  F  V  N  K  D  Q  I  A  K   121

GGATGTGAAGCAGTTCTATGACCAGGCCCTACAGCAGGCCGTGGTGGATGATGACGCCAA  660
 D  V  K  Q  F  Y  D  Q  A  L  Q  Q  A  V  V  D  D  D  A  N   141

CAACGCCAAGGCTGTGGTGAAGACCTTCCACGAGACGCTTGACTGCTGTGGCTCCAGCAC  720
 N  A  K  A  V  V  K  T  F  H  E  T  L  D  C  C  G  S  S  T   161

ACTGACTGCTTTGACCACCTCAGTGCTCAAGAACAATTTGTGTCCCTCGGGCAGCAACAT  780
 L  T  A  L  T  T  S  V  L  K  N  N  L  C  P  S  G  S  N  I   181

CATCAGCAACCTCTTCAAGGAGGACTGCCACCAGAAGATCGATGACCTCTTCTCCGGGAA  840
 I  S  N  L  F  K  E  D  C  H  Q  K  I  D  D  L  F  S  G  K   201

GCTGTACCTCATCGGCATTGCTGCCATCGTGGTCGCTGTGATCATGATCTTCGAGATGAT  900
 L  Y  L  I  G  I  A  A  I  V  V  A  V  I  M  I  F  E  M  I   221

CCTGAGCATGGTGCTGTGCTGTGGCATCCGGAACAGCTCCGTGTACTGAGGCCCCGCAGC  960
 L  S  M  V  L  C  C  G  I  R  N  S  S  V  Y  *               236

TCTGGCCACAGGGACCTCTGCAGTGCCCCCTAAGTGACCCGGACACTTCCGAGGGGGCCA 1020
TCACCGCCTGTGTATATAACGTTTCCGGTATTACTCTGCTACACGTAGCCTTTTTACTTT 1080
TGGGGTTTTGTTTTTGTTCTGAACTTTCCTGTTACCTTTTCAGGGCTGATGTCACATGTA 1140
GGTGGCGTGTATGAGTGGAGACGGGCCTGGGTCTTGGGGACTGGAGGGCAGGGGTCCTTC 1200
TGCCCCTGGGGTCCCAGGGTGCTCTGCCTGCTCAGCCAGGCCTCTCCTGGGAGCCACTCG 1260
CCCAGAGACTCAGCTTGGCCAACTTGGGGGGCTGTGTCCACCCAGCCCGCCCGTCCTGTG 1320
GGCTGCACAGCTCACCTTGTTCCCTCCTGCCCCGGTTCGAGAGCCGAGTCTGTGGGCACT 1380
CTCTGCCTTCATGCACCTGTCCTTTCTAACACGTCGCCTTCAACTGTAATCACAACATCC 1440
TGACTCCGTCATTTAATAAAGAAGGAACATCAGGCATGCTAAAAAAAAAAAAAAAAA     1496
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FIG. 3. Nucleotide sequence of the *tapa-1* gene, showing the amino acid sequence of the open reading frame as well as the eucaryotic consensus translation-initiation site (11) and the polyadenylation site (both underlined). GenBank accession number M33680.

a 238-base-pair 5′ untranslated region that is extremely G+C rich, followed by an ATG that is embedded in a eucaryotic consensus initiation site, CCGCCATGG (11). This is followed by a single open reading frame coding for a protein of 236 amino acids and with a calculated molecular weight of 25,809. The TGA stop codon is followed by 600 untranslated nucleotides, a polyadenylation signal, and a poly(A) tail. The G+C rich portion of the 5′ untranslated region of the gene was removed by digestion at a unique *Nco*I site. The released coding and 3′ untranslated region sequence was then used to probe genomic Southern blots. This probe hybridized under stringent conditions to a single band in genomic DNA digested with several enzymes, indicating that it is not a member of a multigene family (data not shown). Northern analysis showed hybridization of this probe to a single RNA species of approximately 1.6 kilobases in human cells that expressed TAPA-1 but not in U937 cells, which failed to express the protein (data not shown). However, a mouse B-cell lymphoma, 38C13, contained an mRNA that cross-hybridized with the *tapa-1* cDNA probe and was of a similar size. Likewise, the untransfected COS monkey fibroblast cells expressed a cross-hybridizing

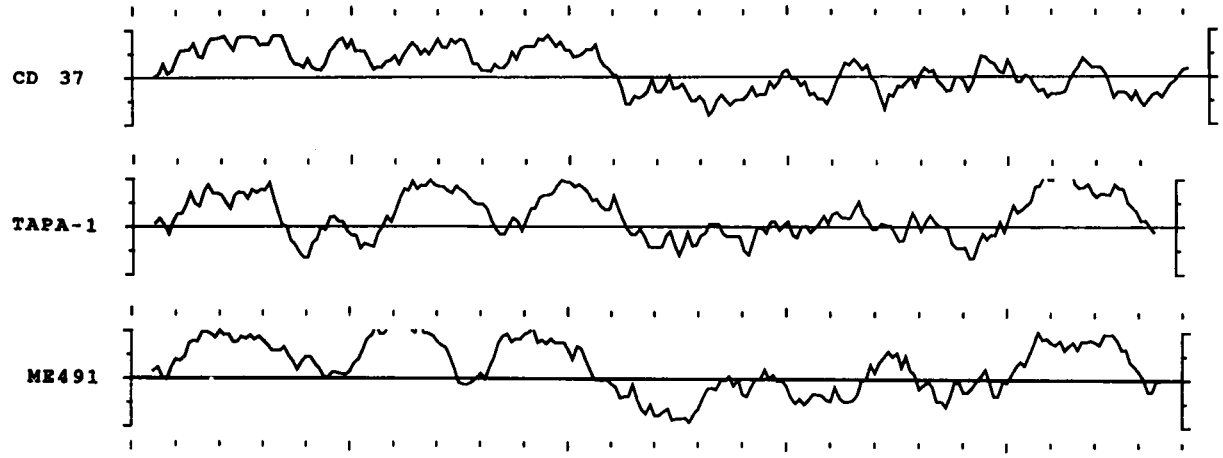

FIG. 4. Hydropathicity plots. TAPA-1, CD37, and ME491 proteins were analyzed as described by Kyte and Doolittle (12). Hydrophobic amino acids are plotted as positive; hydrophilic amino acids are plotted as negative.

mRNA species. Presumably, these distantly related cells contain a message for a protein of similar size which lacks the antigenic determinant recognized by the 5A6 monoclonal antibody.

TAPA-1 contains a potential N-myristoylation site. The amino acid sequence of the TAPA-1 was analyzed by using PROSITE, a dictionary of protein sites and patterns (2). This analysis revealed that the N-terminal glycine is likely to be acylated by myristate. The sequence specificity of myristoyl coenzyme A:protein N-myristoyl transferase requires a core hexapeptide sequence. This hexapeptide contains the required N-terminal glycine in position 1, either asparagine, glutamine, serine, valine, alanine, or leucine in position 2, and serine threonine, cysteine, alanine, asparagine, or glycine in position 5 (27). The N-terminal sequence of TAPA-1 (glycine, valine, glutamic acid, glycine, cysteine) fulfills these criteria and in addition has a basic amino acid, lysine, in position 7, which has also been shown to favor binding of the myristoyl coenzyme A:protein N-myristoyl transferase enzyme (27). Indeed, biosynthetic labeling experiments on whole cells have confirmed that the protein is myristoylated and is hydroxylamine resistant (data not shown).

TAPA-1 defines a new family of transmembrane proteins. The predicted protein sequence of TAPA-1 shown in Fig. 3 contains a single N-linked glycosylation site and is devoid of a signal peptide. Analysis of hydropathicity (12) indicates a protein with four putative membrane-spanning domains (Fig. 4). Analysis of amphipathicity (16) predicts a 50-amino-acid amphipatic helical region between the third and fourth transmembrane domains. A FASTAP (19) search of the protein data bank revealed that TAPA-1 is highly homologous to two other proteins of similar protein backbone size. These proteins are the melanoma-associated antigen ME491 (10) and the leukocyte membrane protein CD37 (6). The amino acid sequence homology (23) of these three proteins to each other is illustrated in Fig. 5. In Fig. 4, the hydrophobicity plots for these three proteins are compared. This analysis illustrates the similarity between these three proteins in the predicted secondary structure of their membrane-spanning regions. However, there are important differences between these proteins. Whereas the TAPA-1 protein contains a potential N-myristoylation site, neither the CD37 nor the ME491 protein contains such a site. In addition, both CD37 and ME491 contain potential N-glycosylation sites in the region between the third and fourth transmembrane domains and are known to be glycosylated. By contract, TAPA-1 contains no N-glycosylation sites in that same region but does contain a potential glycosylation site adjacent to the carboxy-terminal end of the protein.

DISCUSSION

We previously described a strategy to search for cell surface molecules that are involved in growth and growth regulation (30). In this approach monoclonal antibodies are screened for their ability to transmit an antiproliferative signal to cells that express their cognitive cell surface antigens. By analogy, antibodies made against the p185 membrane protein product encoded by the *neu* oncogene have a profound antiproliferative effect on tumor cells or transfectants expressing this gene (7). In this case the oncogene and its protein product were already known before the antibody effects were described. But it is clear that an alternate path to the discovery of this oncogene could have begun with the antiproliferative antibody. We have now applied this strategy and screened hybridomas derived from mice that were immunized with human lymphoma cells and cell lines (Table 1). Monoclonal antibodies that had an antiproliferative effect on the screening cell line OCI-LY8 were further analyzed for the molecular weight and chain composition of their target antigens. About 40% of the antiproliferative antibodies did not precipitate a protein detectable by SDS-polyacrylamide gel electrophoresis. These antibodies might react with very high-molecular-weight antigens that are not solubilized by detergents or do not enter the gel. Alternatively, they might react with nonprotein cell surface structures such as polysaccharides or lipids.

Another group of antiproliferative antibodies precipitated antigens that have been previously characterized and known to have growth-regulating effects such as immunoglobulin and the transferrin receptor. The frequent detection of major histocompatibility complex class I and class II antigens by this assay is interesting. Previous work has shown that antibodies against class I major histocompatibility complex can inhibit the proliferation of activated T cells (26). These observations raise the possibility that major histocompatibility complex molecules have growth regulatory functions in addition to their well-known roles of interacting with T-cell receptors and in antigen presentation. The fact that antibod-

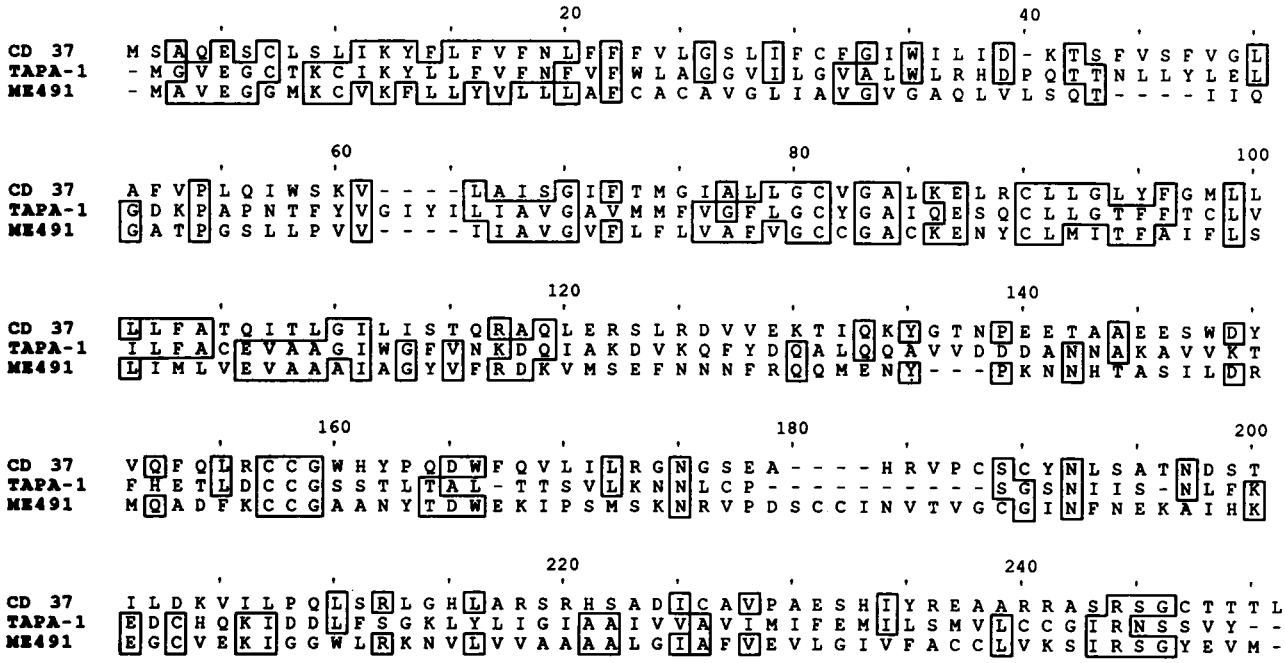

FIG. 5. Amino acid sequence homologies. TAPA-1 was aligned with the CD37 and the ME491 proteins. Identities between any two of the proteins are boxed. Gaps were created by the TULLA (23) program to permit best alignment.

ies against major histocompatibility complex molecules can have direct antiproliferative effects should call for a more cautious interpretation of experiments that use the inhibitory effects of anti-major histocompatibility complex class II antibodies in an attempt to invoke cognate interactions between proliferating cells.

The most interesting antibodies found in the antiproliferative screen were those that recognized new cell surface proteins. These included a 110-kDa molecule repeatedly detected in several independent immunizations and 28- and 26-kDa molecules (Table 1). Morimoto et al. (18) very recently identified a 110-kDa molecule that is the target of an antibody that inhibits antigen-induced T-cell proliferation. It remains to be seen whether the anti-110-kDa molecule antibodies isolated here identify the same antigen.

We do not know what factors in the immunization and screening scheme effect the frequency with which these various target antigens are detected. The prevalence of these molecules on the immunizing cells cannot completely account for the outcome. For example, we have isolated the transferrin receptor only once, even though it is highly expressed on the cell surface and some antibodies against this receptor are known to have antiproliferative effects (24). Recently, a similar approach was used for the isolation of a monoclonal antibody called anti-APO-1, which identifies a 52-kDa cell surface protein that transmits a signal leading to programmed cell death (apoptosis) to several lymphoid cell lines (28). Of the 35 antiproliferative antibodies isolated in the current study, none was directed against this anti-APO-1 molecule.

The 5A6 antibody obtained by immunization and screening on the B-cell line OCI-LY8 was tested on a panel of cell lines for its reactivity and for its antiproliferative effects. This antibody reacted with a large number of cell lines; however, the various cell lines differed in their sensitivity to the antibodies (Table 2). Lymphoid cells were most sensitive to the antiproliferative effect of the antibodies; B cells were effected more than T cells. Although nonlymphoid cells were less effected by the antibody, their reactivity, as measured by flow cytometry, was similar to that of the lymphoid cells. All cell lines that bound the 5A6 antibody expressed a similar immunoprecipable 26-kDa protein regardless of their sensitivity to the antiproliferative activity of the antibody (Fig. 2a). Thus, the expression of the TAPA-1 protein was necessary but not sufficient to render the cells sensitive to the antiproliferative activity of the antibody. The independent role of TAPA-1 in signal transduction will need to be studied by transfecting the gene to cells that do not express either the mRNA or its protein product, such as U937 cells. This experiment may allow a determination of whether the TAPA-1 molecule isolated from a sensitive cell can serve as a target for the antiproliferative effect of the antibody. If associated molecules are required to transmit antiproliferative signals, then the recipient cells will need to provide them. By analogy with other lymphoid membrane molecular complexes (3, 25), it is possible that the TAPA-1 protein is associated with other molecules that are critical for transduction of the antiproliferative effect of the antibodies. Preliminary experiments (Takahashi et al., submitted for publication) have indicated a noncovalent association between the TAPA-1 protein and several other proteins, including the Leu-13 antigen (4). It has been previously shown that antibodies to Leu-13 inhibit the mitogenic effect of anti-CD3 antibodies on T cells (4). Thus, at least one of the proteins associated with TAPA-1 can also transmit an antiproliferative signal.

The mechanism of antiproliferative action of the 5A6 antibody against TAPA-1 is not clear. Our experiments have failed to demonstrate either Ca^{2+} flux or apoptosis in response to the antibody. By analogy, with the *neu* oncogene, TAPA-1 might be a receptor for a yet-to-be-discovered growth factor. However, unlike *neu*, the sequence of TAPA-1 does not suggest any homology to known growth factor receptors. We have not yet determined whether antibodies

reactive with other sites on the TAPA-1 molecule will induce similar antiproliferative effects to the 5A6 antibody. The mechanism of antiproliferative effect of the anti-Neu antibodies seems to be the removal of the p185 epidermal growth factor receptor analog from the cell surface and the deprivation for the cell of its receptor. If physical removal is the mechanism of antiproliferative effect of the 5A6 antibody, then antibodies against other determinants on the TAPA-1 molecule should also be antiproliferative.

An important finding in the current study was the homology between TAPA-1, CD37, and ME491. Together, these three proteins define a new family of membrane molecules implicated in the control of cell proliferation. The CD37 molecule is highly expressed on human B cells and to a lesser extent on T cells and myeloid cells. Anti-CD37 antibodies have been shown to inhibit the mitogenic effect of CD20 plus B-cell growth factor on B cells but to stimulate the mitogenic effect of anti-immunoglobulin antibodies on these cells (14). ME491 is expressed during the early stages of melanoma but is down-regulated during the later stages of melanoma, which are more aggressive and adaptable to tissue culture. It has therefore been suggested that the ME491 antigen plays a role in growth regulation (10).

All three of these proteins lack an amino-terminal signal peptide, a feature of many integral membrane proteins (22). Integration of such proteins into the membrane has been suggested to occur spontaneously based on their hydrophobic nature. Alternatively, these proteins may have an internal signal sequence. Most transmembrane proteins that do not have a cleavable signal peptide are thought to be oriented with their amino terminus toward the cytoplasm. The hydropathicity analysis (12) indicates that all three proteins have three contiguous putative transmembrane regions followed by an amphipathic hydrophilic region (Fig. 5). Two of the proteins, TAPA-1 and ME491, contain an additional putative transmembrane region near their carboxy termini. The three genes code for a protein backbone of a very similar size. Whereas TAPA-1 is probably not glycosylated, although it contains a potential N-glycosylation site, the other two proteins are glycosylated at either one or more of three potential glycosylation sites, all located in their hydrophilic domain. Such glycosylation usually marks the portion of transmembrane proteins that is external to the cell. TAPA-1 is likely to be myristoylated at its N-terminal glycine; this is not the case for CD37 or ME491. Preliminary labeling experiments have shown that TAPA-1 protein is myristoylated. Additional studies are in progress to determine whether this myristoylation occurs at the N terminus.

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ADDENDUM IN PROOF

It has been brought to our attention that the original reported sequence of human CD37 was incorrect, and as a result, the reading frame was terminated 16 amino acids before the carboxy-terminal end. The revised CD37 sequence (B. J. Classon and A. F. Williams, J. Exp. Med., correction in press), like the other members of the TAPA-1 family, has four transmembrane domains.

In addition, SM23, a surface antigen of *Schistosoma mansoni*, has recently been reported (M. D. Wright, K. J. Kenkle, and G. F. Mitchell, J. Immunol. **144**:3195–3200, 1990) and is a member of the TAPA-1 family.

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