

THE ISOLATION AND SEQUENCE OF A NOVEL GENE FROM A HUMAN FUNCTIONAL T CELL LINE

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The study of T cell activation, proliferation, and effector function has been facilitated by the availability of long term in vitro cultures of clonal T cell lines (1-3). Growth of such cell lines depends on periodic stimulation with growth factors, including IL-2, and with antigen that is recognized by the Ti/CD3 cell surface complex. It is believed that these IL-2-dependent, antigen-specific T cell lines, which function in vitro as helper or cytolytic cells, are normal counterparts of the T lymphocytes that perform these important effector and regulatory roles in vivo.

To identify genes and gene products involved in the molecular mechanisms of T cell activation and effector function, we have begun a systematic search for cDNA clones expressed in functional cytolytic and Th cell lines but not in cell lines of other lineages. Here we report the structure of two overlapping cDNA clones that represent a gene, designated 519, that is expressed in 10 functional T cell lines but not in 14 established tumor lines, including 6 T cell tumor lines. We also show that the expression of 519 mRNA in PBL is increased more than 10-fold by mitogenic or antigenic stimulation. This increase is first detected 3-5 d after initiation of the culture. It is thus possible that the 519 gene product is involved in the activation and subsequent proliferation and/or differentiation of resting T cells. The data also show that functional T cell lines express gene products that are not readily detected in long term T cell tumor lines.

Materials and Methods

Cell Lines. The characterization and growth of the functional T cell lines and clones have been described (4, 5).

Construction of cDNA Libraries. Construction of a cDNA library enriched for T cell-specific sequences was as described (6, 7) with minor modifications. Briefly, total cytoplasmic RNA was extracted from the CD8⁺, HLA-A2-specific functional cytolytic T cell line AH2 (4) by NP-40 lysis, and poly(A)⁺ mRNA was prepared by oligo(dT) chromatography (8). Single-stranded cDNA was synthesized using reverse transcriptase primed with

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oligo-dT₍₁₂₋₁₈₎ in the presence of actinomycin D. To enrich for cDNA sequences specific for functional T cell lines, ~1 μ g of total cDNA was then hybridized to a c_{ot} of >2,000 mol·sec/liter with 10 μ g of oligo(dT)-selected mRNA extracted from the EBV-transformed B cell line LB. ~100 ng of unhybridized cDNA was recovered with a yield of 65–70%. We thus estimate that the unhybridized cDNA was enriched approximately sevenfold for T cell-specific sequences. This cDNA was made double stranded and C-tailed and annealed with G-tailed pUC9 (Pharmacia Fine Chemicals, Piscataway, NJ) as described (6, 7), except that 10 μ g oligo(rA) (Pharmacia Fine Chemicals) was used as carrier. The annealed DNA was then used to transform competent *Escherichia coli* HB101 (Bethesda Research Laboratories, Gaithersburg, MD). The final subtracted cDNA library consisted of 20,000 colonies.

To isolate (near) full length cDNA clones of the genes identified in the subtracted library we constructed a cDNA library using total cytoplasmic poly(A)⁺ RNA from the AH2 cell line. First and second strand synthesis of the cDNA was according to the method of Gubler and Hoffman (9). This double-stranded cDNA was then cloned in the λ gt10 phage using Eco RI linkers as described by Huynh et al. (10). After in vitro packaging using a commercial packaging extract (Amersham Corp., Arlington Heights, IL) and plating on *E. coli* C600, we obtained 1.5×10^7 phage plaques. ~2% of these plaques were due to recombinant phages capable of replicating in *E. coli* C600 Hf1⁺. Screening of the library was performed after plating 10^5 recombinant phages on *E. coli* C600 Hf1⁺.

Screening with Subtracted cDNA Probes. Radioactive subtracted probes were prepared as described (6, 7). Briefly, cDNA from AH2 cells labeled to high specific activity (10^8 cpm/ μ g) with [³²P]dCTP was subtracted twice with RNA from LB cells. The final single-stranded probe (2×10^7 cpm) was used to screen four large nitrocellulose filters, each containing 5,000 colonies of the subtracted AH2-LB cDNA library. Hybridization was in 3.5 ml aqueous buffer containing 1 M Na⁺ as described (6, 7) at 68°C for 20 hrs. Filters were washed twice in 2 \times SSC at 65°C for 30 min.

Isolation of Cytoplasmic RNA. 10^9 cells were spun down and washed once in ice-cold PBS. Cells were resuspended in 10 ml ice-cold lysis buffer (0.14 M NaCl, 0.01 M Tris, pH 8.0, 1.5 mM MgCl₂) and then 10 ml of ice-cold lysis buffer containing 0.8% NP-40 was added. The cell suspension was placed on ice for 2 min before pelleting the nuclei at 800 g for 5 min. The supernatant was made 1% in SDS and 5 mM in EDTA, and extracted twice with phenol at 65°C and once with chloroform. The aqueous phase was then made 0.3 M in sodium acetate and the RNA was precipitated with 2.2 volumes of ethanol at –20°C overnight. The RNA precipitate was then pelleted at 2,500 g for 30 min. After drying the pellet, the RNA was resuspended in 0.1 ml H₂O.

Isolation of Whole Cellular RNA. This was done according to the guanidinium isothiocyanate method described by Chirgwin et al. (11).

Northern Blot Analysis. RNA was separated on gels containing 1% agarose, 3% formaldehyde, 1 mM EDTA in 20 mM sodium borate buffer, pH 8.3. RNA samples were made 50% formamide, 6% formaldehyde, 2 mM EDTA, and 10% glycerol in 20 mM sodium borate buffer, pH 8.3, and heated to 55°C for 15 min before loading. Gels were run for 8 h at 80 V, and the RNA was transferred to Genetran nylon filters (Plasco, Woburn, MA) by blotting the gel overnight.

Hybridization of Northern Blots. Whole plasmids were labeled with ³²P by nick translation. DNA fragments were labeled with Klenow enzyme using random hexamer priming (12). The nylon filters were prehybridized for 4 h at 42°C and hybridized at the same temperature overnight with 3×10^6 cpm of probe per ml of hybridization buffer. Prehybridization and hybridization buffers were 3 \times SSC, 50% formamide, 1% SDS, 0.5 mM sodium pyrophosphate, 0.1 M sodium phosphate, pH 7.0, 2.5 \times Denhardt's solution, 100 μ g/ml salmon sperm DNA and 2.4% dextran sulphate. Filters were washed twice for 20 min in 2 \times SSC, 0.1% SDS at room temperature, followed by a 30 min wash in 0.2 \times SSC, 0.1% SDS at 60°C.

Sequence Analysis of cDNA Clones. cDNA clones were sequenced using the method of Maxam and Gilbert (13). The sequence of the 5' end of clone 519.11 was verified using the chain termination method of Sanger (14). The DNA sequence of the two overlapping

clones AH2-519 and 519.11 and the predicted protein sequence were compared against published sequences compiled in the GenBank and NBRF databases on a VAX 11/785 computer using the FASTP program (15).

In Vitro Transcription and Translation. After subcloning the cDNA insert of phage 519.11 into the Eco RI site of the plasmid vector pGem-1 (Promega Biotec, Madison, WI), RNA was transcribed from both strands using either SP6 or T7 polymerase. The transcription reactions were performed in the presence of 500 μ M diguanosine-triphosphate, G(5')ppp(5')G, and 50 μ M dGTP (16). Reactions were terminated by digestion of the DNA template with RNase-free DNase I (RQ DNase, Promega Biotec) and the RNA was purified by phenol and chloroform extractions, followed by G-50 chromatography and ethanol precipitation. RNA from both strands was then translated in vitro (0.3 μ g each) using a commercial rabbit reticulocyte extract (Promega Biotec) in the presence of [³⁵S]methionine and the translation products were analyzed using a 20% polyacrylamide gel prepared and run according to Laemmli (17).

Genomic Southern Blot Analysis. Genomic DNA was digested with restriction enzymes in the presence of 4 mM spermidine. The digested DNA samples were made 2.5% Ficoll, and 10 μ g DNA was size separated on a 0.7% agarose gel in Tris-acetate-EDTA buffer for 20 h at 40 V. The DNA was transferred to nitrocellulose paper (Schleicher & Schuell, Inc., Keene, NH) by blotting, using 20 \times SSC for 24 h. The filter was then baked at 80°C in a vacuum oven. After an overnight prehybridization at 65°C, the nitrocellulose filter was hybridized overnight at 65°C with the cDNA insert of phage 519.11 (5×10^6 cpm/ml), labeled with ³²P using the random hexamer priming method (12).

Results

Isolation of Specific cDNA Clones from a Subtracted Library. To isolate cDNA clones representing genes expressed specifically in functional T cell lines we screened the AH2-LB, T-B-subtracted cDNA library with a highly radioactive subtracted cDNA probe (T*-B cDNA probe; sp act, 10⁸ cpm/ μ g) prepared from RNA extracted from the cytolytic T cell line AH2. The cDNA was subtracted with RNA from the EBV-transformed B cell line LB. After two rounds of screening we identified 53 positive colonies. To eliminate those clones representing rare abundance mRNA sequences expressed in T and B cells as well as other clones that were not efficiently removed by the subtraction procedure, we prepared a subtracted B*-B cDNA probe. We used cDNA prepared from LB cells that was subtracted under standard conditions with its template RNA. This allowed us to eliminate 17 clones that hybridized to this B*-B probe. Finally, eight clones with inserts >300 bp were selected, seven of which cross hybridized. The clone with the longest insert in this group of seven (designated AH2-519; 382 bp including 34 A residues at its 3' end; see Fig. 1) was selected for further study.

Cell Type-Specific Expression of 519 RNA. We used the cDNA insert of clone AH2-519 as a probe on Northern blots containing RNA from a variety of leukemic or virally transformed cell lines of different cell lineages and several independently isolated functional T cell lines. As shown in Fig. 2a, we detect 519 mRNA expression only in functional T cell lines, including five cytolytic T cell lines AI5.1, AI1.1, AJY (Fig. 2a), CCD, and AH2 (data not shown). The expression of gene 519 is not restricted to functional cytolytic T cells since the five functional Th cell lines AJ13.1, AJ8.1, APL2, APL1 (Fig. 2a), and ALP2 (not shown) contain 519 mRNA at levels comparable to the functional cytolytic T cell lines. The 519 mRNA is detected as a diffuse band with an average length of ~900 bp. The diffuse appearance of the 519-specific RNA may be due to

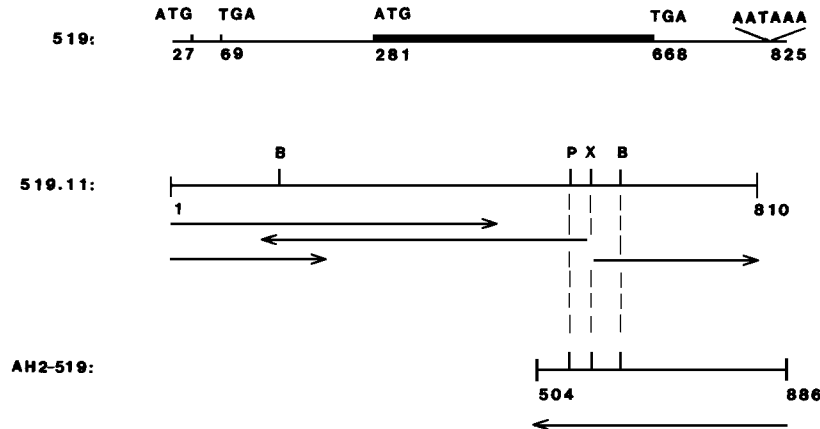


FIGURE 1. Schematic representation of 519 cDNAs. The bottom line depicts the 382-bp long cDNA clone AH2-519 isolated from the AH2-LB subtracted cDNA library. The middle line depicts the longer cDNA clone 519.11 isolated from a λ gt10 cDNA library made from AH2 cells. The two clones overlap from bp 504 to 810. Numbering starts at the most 5' nucleotide of clone 519.11. Key restriction sites used in preparing DNA fragments for sequencing are indicated (B, Bgl II; P, Pst I; X, Xba I). Arrows indicate the extent and direction of sequencing by the method of Maxam and Gilbert (13). The sequence of the 5' end of clone 519.11 has been verified using the chain termination method (14). Clone AH2-519 is the longest of a group of seven crosshybridizing clones. The other six clones have been sequenced using the chain termination method and have been shown to correspond to different short DNA fragments overlapping clones AH2-519 and 519.11. The top line is a composite diagram of the structure of the 519 mRNA based on the sequence of the two overlapping clones AH2-519 and 519.11. The thin line represents untranslated regions, the thick line represents the single long open reading frame coding for a 129-amino-acid protein. The initiation and termination codons of the potential short open reading frame starting at nucleotide 27 are also indicated, as is the single polyadenylation signal starting at nucleotide 825.

incomplete denaturation under the conditions used for sample preparation before loading the gel. Shorter exposures of the autoradiogram shown in Fig. 2a do not reveal evidence for the presence of more than one size of mRNA. As expected, we do not detect 519 mRNA in five EBV-transformed B cell lines (TOTO, PALLY, LCL-721, DAUDI, [Fig. 2a], and LB [not shown]). In addition, three nonlymphoid tumor lines, the promyelocytic leukemia line HL60, the myelomonocytic leukemia line U937, and the erythroleukemia line K562, are negative for 519 expression as well. Surprisingly, we do not detect 519 RNA expression in six established T cell tumor lines (MOLT-3, HUT78, HSB, HPB-ALL [Fig. 2a], Jurkat, and MOLT4 [not shown]). Thus, among the in vitro-derived tissue culture cells tested, the expression of 519 RNA is restricted to nontransformed, functional T cell lines.

To investigate whether other nontransformed cells express 519 RNA, we hybridized a Northern blot containing RNA from a variety of normal tissues (a gift of Drs. Thomas Reynolds and Jeffrey Sklar, Stanford University School of Medicine). As can be seen in Fig. 2b, none of the tissues tested (tonsil, placenta, lung, liver, skeletal, and smooth muscle) express 519 RNA. In addition a human fibroblast cell line KB is also negative. To control for the quality of the RNA samples on the blots shown in Fig. 2, a and b, both blots were hybridized with

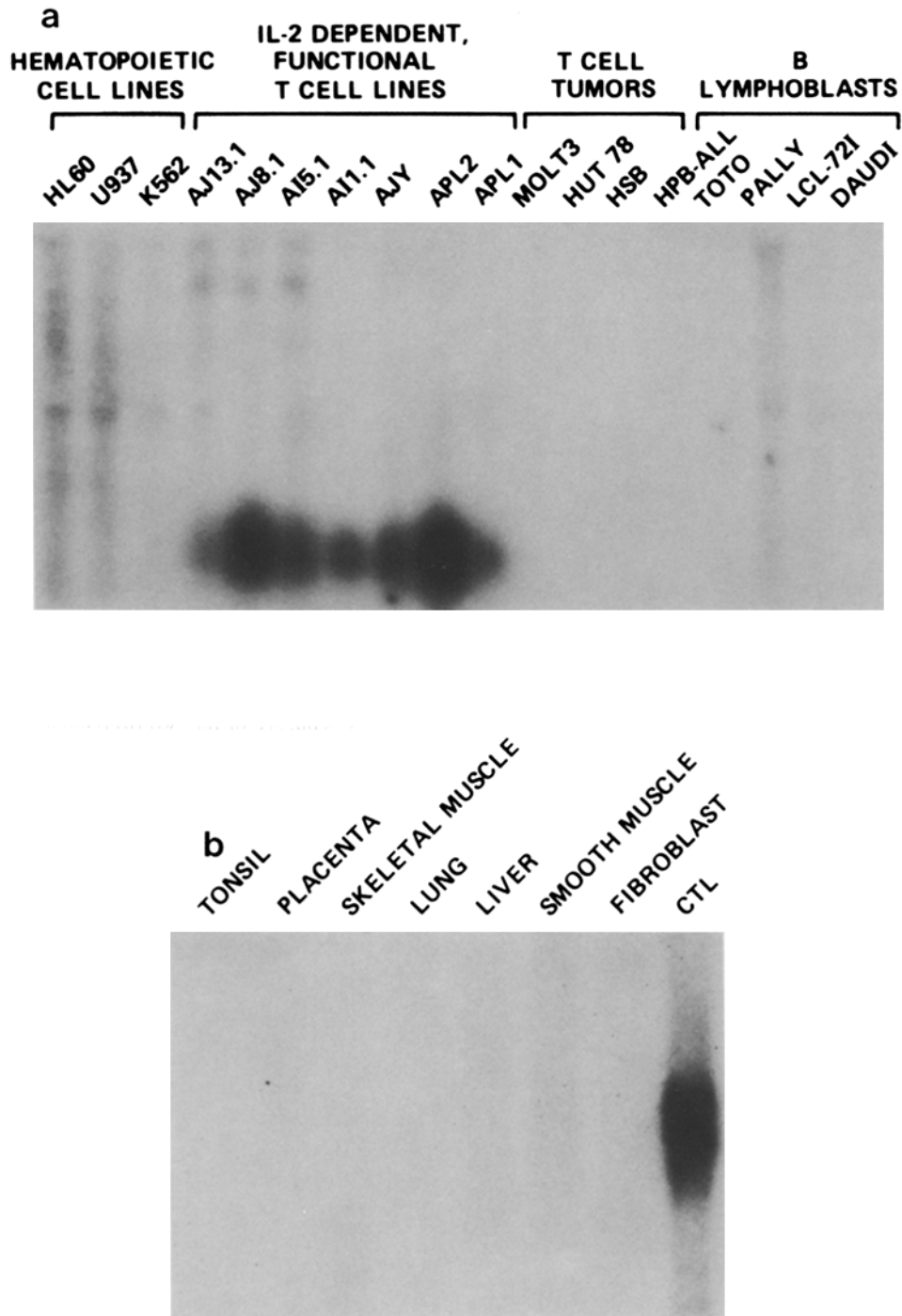


FIGURE 2. Expression of 519 RNA. Northern blots were prepared as described and probed with the cDNA inserts of clone AH2-519. Identical results were obtained when the longer insert of clone 519.11 was used as a probe. The center of the broad 519 band was determined to be between 800 and 900 bp, using the rRNA bands on the ethidium bromide-stained gel as markers. Autoradiograms were exposed for 1–2 d at -70°C using an amplifying screen. (a) Northern blot using 20 μg total cytoplasmic RNA of the indicated tissue culture cell lines. (b) Northern blot using 4 μg total poly(A)⁺ RNA of the indicated tissues. The fibroblast lane contains RNA from the cell line KB. The CTL lane contains 20 μg total cytoplasmic RNA from line AJY as in a.

O A-1 P-1 A-3 P-3 A-5 P-5 A-7 P-7

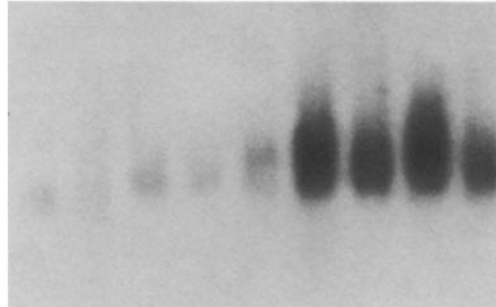


FIGURE 3. Induction of 519 RNA. Northern blot containing 10 μ g of total RNA extracted from freshly isolated PBL (lane O) or from PBL cultured with the mitogen PHA (P) or the lymphoblastoid cell line JY as alloantigen (A). RNA was extracted 1, 3, 5, or 7 d after the start of the culture. The blot was hybridized with the insert of clone AH2-519.

an MHC class I cDNA probe. We detect a signal in all lanes of both blots, except those containing RNA from the K562 cell line that is known not to express MHC class I antigens. Since the strength of the signal does not vary more than fourfold, the differences in 519 expression is not due to differences in the quality or quantity of the RNA samples tested. The quality of K562 RNA was judged to be equivalent to the other samples from the ethidium bromide-stained agarose gel.

These results show that the expression of the 519 gene is not a characteristic of all nontransformed cells, but is restricted to IL-2-dependent, antigen-driven functional T cell lines.

The Inducible Expression of 519 RNA. The restricted expression pattern of 519 RNA suggests that the expression of the 519 gene is regulated by the periodic addition of IL-2 and antigen to the functional T cell lines. We thus measured 519 RNA expression in cultures of normal PBL, stimulated for different periods of time with antigen in a mixed lymphocyte reaction or stimulated with the mitogen PHA. Both kinds of stimulation lead to the early synthesis and release of IL-2 (18). As shown in Fig. 3, freshly derived PBL express 519 RNA (lane O). No change in expression of 519 RNA is detected in PBL grown for 1 d or 3 d in a mixed lymphocyte culture (lanes A-1 and A-3) or in the presence of PHA (lanes P-1 and P-3). However, in both types of cultures a dramatic increase in the expression of 519 RNA can be detected 5 d after the start of the culture (lanes A-5 and P-5). Cultures tested at day 7 do not reveal a further increase in 519 RNA expression (lanes A-7 and P-7). Further time points were not tested. To determine whether the expression of 519 RNA is inducible in other activated lymphocytes, we activated splenic B cells with fixed *Staphylococcus aureus*. After 3 d, at the time of maximum blast formation the cells were harvested and depleted of activated T cells by treatment with anti-CD2 and anti-CD3 monoclonal antisera and complement. RNA extracted from the treated cells did not hybridize with the 519 probe.

Thus, these experiments show that the expression of the 519 gene is regulated in normal T cells, but not in normal B cells, by mitogenic or antigenic stimulation.

Isolation and Sequence Analysis of a Full Length 519 Clone. Using the short

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                20                40                60
CCTGGGCCCTCCTGCTCCTTGCAGCCATGCTCCTGGGCAACCCAGCCCCTGCCTCCGCAT
                80                100               120
CTGCGTGGTGAAGCCATTGGCCTCATCGGTGGATCTGCGTTTCCTCGGGCCCCACTGT
                140                160               180
CTAGGATTGTGCGGGCTGGTGAGAGAACAAGATCTCTCCGTGTTCAAGGCAGACTTCC
                200                220               240
TGCCCCCTGCACCCCTGCTCTCTCCGGGCCTTGAGGTGAGTGTGAGCCCCAAGGGCAAGA
                260                280               300
ACACTTCTGGAAGGAGAGTGGATTGGCTGGCCTCTGGATGGAAGTCTGGTCTTCTC
MetGluGlyLeuValPheSe

                320                340               360
TCGTCTGAGCCCTGAGTACTACGACCCGGCAAGAGCCACCTGCGTGATGGGGAGAAATC
rArgLeuSerProGluTyrTyrAspProAlaArgAlaHisLeuArgAspGlyGluLysSe
                10                20

                380                400               420
CTGCCGTGCGGGCAGGAGGGCCCCAGGGTGACCTGTTGACCAAAACACAGGAGCTGGG
rCysProCysGlyGlnGluGlyProGlnGlyAspLeuLeuThrLysThrGlnGluLeuGl
                30                40

                440                460               480
CCGTGACTACAGGACCTGTCTGACGATAGTCCAAAACTGAAGAAGATGGTGGATAAGCC
yArgAspTyrArgThrCysLeuThrIleValGlnLysLeuLysLysMetValAspLysPr
                50                60

                500                520               540
CACCCAGAGAAGTGTTCCAATGCTGCGACCCGGGTGTAGGACGGGGAGGTACAGATG
oThrGlnArgSerValSerAsnAlaAlaThrArgValCysArgThrGlyArgSerArgTr
                70                80

                560                580               600
GCGCGACGTCTGCAGAAATTTTCATGAGGAGGTATCAGTCTAGAGTTATCCAAGGCCTCGT
pArgAspValCysArgAsnPheMetArgArgTyrGlnSerArgValIleGlnGlyLeuVa
                90                100

                620                640               660
GGCCGGAGAAACTGCCAGCAGATCTGTGAGGACCTCAGGTTGTGTATACCTTCTACAGG
lAlaGlyGluThrAlaGlnGlnIleCysGluAspLeuArgLeuCysIleProSerThrGl
                110                120

                580                700               720
TCCCCTCTGAGCCCTCTCACCTTGTCTCTGTGGAAGAAGCACAGGCTCCTGTCTCAGATC
yProLeuEnd
                740                760               780
CCGGAAACGTCAGCAACCTCTGCCGGCTCCTCGCTTCCTCGATCCAGAATCCACTCTCCA
                800                820               840
GTCTCCCTCCCCTGACTCCCTCTGCTGTCTCCCTCCTCAGGGGAATAAAGTGTCAAGCA
                860                880
AGATTTTAGCCGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

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FIGURE 4. DNA sequence of 519 mRNA. The DNA sequence shown is a composite of the DNA sequence of the two overlapping clones AH2-519 and 519.11. The predicted amino acid sequence of the 519 gene product is shown beneath the DNA sequence from nucleotide 281 to 668. The polyadenylation signal starting at nucleotide 825 is underlined.

AH2-519 clone as a probe we screened a λ gt10 cDNA library prepared from total cytoplasmic poly(A)⁺ RNA from AH2 cells. One clone, 519.11, was selected because the length of its insert is similar to the size of the 519-specific mRNA, as determined by Northern analysis. We determined the DNA sequence of both the short AH2-519 and the longer 519.11 clones according to the strategy outlined in Fig. 1. The two clones overlap from 504–810 bp. The sequence from both clones allowed us to deduce the sequence of the 519 mRNA to be at least 853 nucleotides, exclusive of the poly(A) tail and possibly some nucleotides at the 5' end not included in the 519.11 clone (Fig. 1, *top*). The DNA sequence, as derived from these two overlapping clones, is shown in Fig. 4. Inspection of this sequence revealed the following: (a) comparison of the DNA sequence or the predicted amino acid sequence revealed no homology with published sequences compiled in the GenBank and NBRF databases; (b) there is a single long open reading frame coding for a protein of 129 amino acids with a predicted molecular weight of 14.6×10^3 , starting at the ATG codon at position 281 and terminating at the TGA codon at position 668. The presumed initiation ATG codon is the

second ATG in the sequence. The first ATG at position 27 is followed by an in-frame stop codon at position 69. It is noteworthy that the ATG sequence at position 281 has little homology to the Kozak consensus sequence for eukaryotic translation initiation sites (TGGATGG versus the consensus ACCATGG, reference 19); (c) a single polyadenylation signal is present at position 825 (AATAAA) followed by a stretch of 34 A residues starting at position 853; (d) a hydropathy plot from the predicted amino acid sequence (Fig. 5) shows that the predicted protein is very hydrophilic and although it contains some hydrophobic regions, there does not appear to be any region long enough to constitute a membrane-spanning domain. The amino acid sequence of the NH₂-terminal part of the predicted protein does not strongly resemble the typical sequence found in eukaryotic signal peptides (20). Taken together, this suggests that the 519 gene product is not a membrane bound or secreted protein; (e) the overall charge of the protein is likely to be positive since it contains more basic than acidic residues (22 Arg and Lys residues vs. 14 Glu and Asp residues); and (f) no potential *N*-linked glycosylation sites are present in the predicted sequence.

In Vitro Transcription and Translation of 519.11. To unequivocally determine the coding strand and the translational capacity of clone 519.11, we subcloned the 519.11 insert into the vector pGem-1 and transcribed both strands using either SP6 or T7 polymerase (16). Both RNA preparations were then translated in vitro in a rabbit reticulocyte extract. Fig. 6 shows that the sense RNA containing the poly(A) tract at its 3' end is translated into a protein with an apparent molecular weight of ~15,000 (lane +, filled triangle), in close agreement with the predicted molecular weight of 14.6×10^3 . No specific translation products are detectable using the antisense RNA strand as a template in the rabbit reticulocyte extract (lane -). The open triangle in Fig. 6 shows the globin molecules translated from the globin mRNA present in the reticulocyte extract. We noticed that the in vitro-generated RNA is inefficiently translated compared with either globin RNA or the Brome Mosaic virus RNA supplied as positive control RNAs with the reticulocyte extract. Mixing experiments suggest that this is not due to impurities in the 519 RNA preparation. We suggest that the poor translational efficiency is related to the presence of the short open reading frame that can potentially code for a 14-amino-acid long peptide preceding the long reading frame and/or to the poor match of the sequence surrounding the ATG codon at position 281 with the consensus sequence for eukaryotic translation initiation sites (19). Inefficient translation in vitro has been reported (21, 22) for two other genes containing upstream ATG codons.

Southern Analysis of Genomic DNA. We hybridized the ³²P-labeled 519.11 fragment with Southern blots containing genomic DNA. The DNA samples were prepared from germline cells (sperm) or from functional CTL or EBV-transformed cell lines established from PBL of two individuals (Fig. 7, AK and PW) or from the tumor line HUT78 (data not shown) and were digested with Eco RI or Bam HI (Fig. 7) or Hind III (results not shown). The results in Fig. 7 show only two 519-related bands. The Hind III digest showed three 519-related bands. No differences were detected among the DNA samples extracted from different cell types of the different individuals or from the HUT78 tumor. We conclude from this that the 519 gene is present in, at most, two copies per

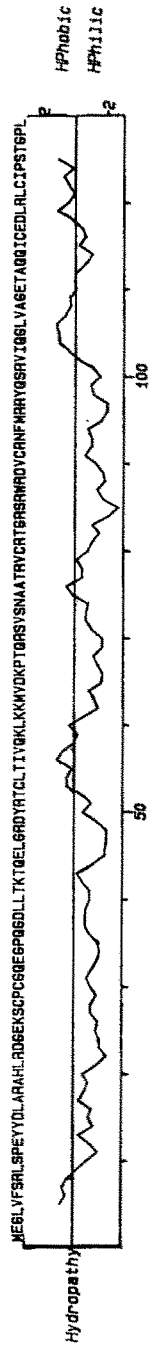


FIGURE 5. Hydropathy plot of the predicted 519 protein. The predicted amino acid sequence of the 519 protein is shown in one-letter code on the top line. The hydropathy plot was generated using the pepplot program in

the University of Wisconsin Genetics Computer Group software package on a VAX 11/785 computer.

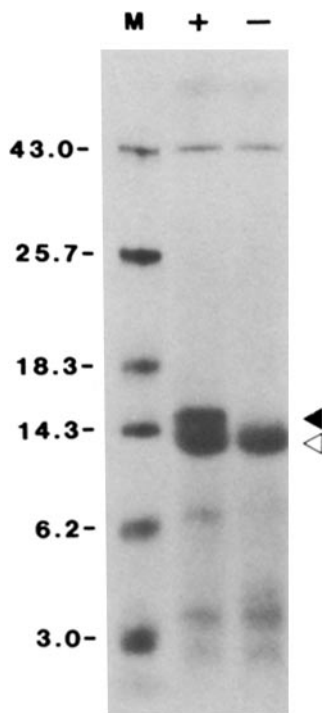


FIGURE 6. In vitro translation of 519 RNA. Sense and antisense RNA was prepared from the insert of clone 519.11 subcloned into the plasmid pGem-1 as described under Materials and Methods. Each RNA (0.3 μ g) was translated in vitro in a rabbit reticulocyte extract in the presence of [35 S]methionine. The translation products were analyzed on a 20% polyacrylamide gel according to Laemmli (17). +, sense strand RNA. -, antisense RNA. The closed triangle denotes the position of the specific 519 translation product; the open triangle shows the position of globin protein translated from the endogenous globin mRNA present in the extract. M, 14 C-labeled molecular weight standards $\times 10^{-3}$.

haploid genome and that the absence of 519 RNA expression in B lymphoblasts or T cell tumor lines is not due to gross rearrangement or loss of the 519 gene.

Discussion

In this paper we describe the isolation and characterization of two overlapping cDNA clones representing a gene, designated 519, that is expressed in IL-2-dependent, antigen-driven functional T cell lines but not in a variety of other cell types, including long-term T cell tumor cell lines. In addition, we find that the expression of 519 is induced at least 10-fold in PBL by antigenic or mitogenic stimulation.

Stimulation of T cells with growth factors and antigen or with mitogen, induces the expression of a number of genes, some of which code for molecules related to the development of effector function. The best studied are the genes coding for IL-2 (18, 23, 24) and its receptor (25, 26) and a class of genes coding for serine proteases thought to be involved in cytolytic effector function (27-29). The inducible expression of the protooncogene *c-myc* and other genes of unknown function has also been reported (25, 29). In addition, a number of cell

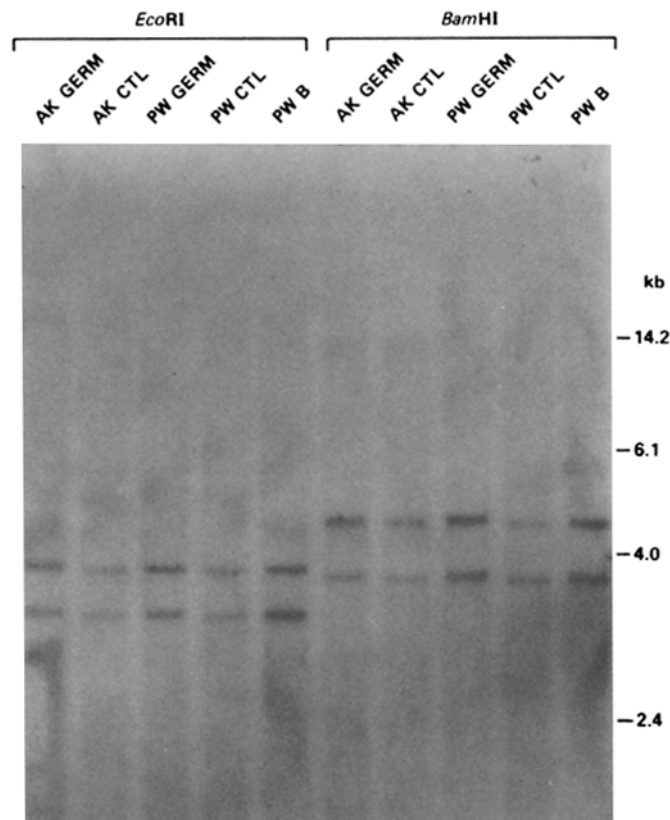


FIGURE 7. Southern analysis of genomic DNA. DNA samples were extracted from two individuals (*AK* and *PW*) using either germline cells (*germ*) or cytolytic T cells (*CTL*) or EBV-transformed B cell lines (*B*) established from PBL. DNA samples (10 μ g) were digested with *Eco* RI or *Bam* HI. The Southern blot was hybridized using the insert of clone 519.11. The size markers indicated at the right in kilobases were determined using a 123-bp ladder (Bethesda Research Laboratories).

surface proteins or antigens have been described that can be detected on stimulated T cells but not on resting T cells. Some molecules such as the transferrin receptor, the IL-2-R, or the insulin receptor can be detected within 24 h after stimulation (reviewed in reference 30), while other molecules such as HLA-DR, Act I, LDA₁, and VLA-1 are not detected until days or weeks after stimulation (30–32).

The technique of subtractive hybridization appears to be well suited for a systematic search for cell type-specific molecules (6, 7, 33) because it provides an approach to isolating important molecules that is not biased towards molecules present in specific cellular compartments. This is in contrast to an alternative method of generating antisera against cell type-specific cell surface molecules.¹

The restricted expression pattern of the 519 gene raises several interesting questions on the possible function of the 519 gene product and the regulation of 519 gene expression. The induction of 519 RNA in PBL stimulated by antigen

¹ McIntyre, B. W., K. Kadiyala, C. Clayberger, A. M. Krensky, and P. Parham. Selective absorption of polyclonal antisera identifies novel T cell differentiation antigens. Submitted for publication.

or by PHA only after 5 d in culture suggests that the 519 gene product is not involved in the initial stages of T cell activation such as the transition from the G₀ to G₁ phase of the cell cycle or the expression of IL-2 and its receptor shortly after stimulation. In addition, the expression of 519 RNA in both cytolytic and Th lymphocytes indicates that the 519 gene product is not involved in the specialized helper or cytolytic functions per se. Since 519 RNA is detected only in activated, nontransformed T cells and not in transformed T cells, the 519 gene product may be involved specifically in some aspect of the growth of and/or differentiation of normal growth factor-dependent T cells. The 519 protein is likely to act intracellularly, since the predicted amino acid sequence of the 519 protein does not support the notion that the 519 protein is secreted or membrane bound to function as a lymphokine or a membrane receptor molecule.

We do not know whether the nontransformed cell types from which the long-term T cell tumor lines used in this study were derived, express the 519 RNA. If so, it suggests a nonrandom loss of 519 gene expression associated with transformation. That raises the possibility that the lack of expression of the 519 gene in certain T cells is a prerequisite for the transformed phenotype. Further detailed studies of the expression of the 519 gene in normal tissues and their transformed counterparts are necessary to clarify this point.

We will use the 519.11 clone to isolate genomic clones containing the 519 gene and determine its exon/intron structure. It will then be possible to determine the *cis*-acting DNA sequences in the 519 locus that are involved in the T cell-specific expression of this gene and its inducibility by IL-2 or mitogen. This has recently been studied (24) for the human IL-2 gene. However, in contrast to the induction of 519, the IL-2 gene is induced within hours after addition of mitogen to the T cell cultures. It will thus be interesting to determine whether the expression and inducibility of 519 and IL-2 is regulated through different DNA sequences.

In addition to the 519 gene described here, we have recently isolated three other noncrosshybridizing cDNA clones that are expressed in functional human T cell lines but not in T cell tumor lines. Similarly, we have isolated a mouse cDNA clone, pJJ32, that is expressed in normal mouse T and B lymphocytes and in a variety of B cell tumor lines, but not in the four T cell tumor lines tested (reference 33, and our unpublished observations). Elsewhere we have shown¹ that the IL-2-dependent antigen-specific T cell lines express many as yet uncharacterized cell surface proteins when compared with T cell tumor lines. These findings strongly suggest that normal nontransformed T lymphocytes express a multitude of genes that can not be readily identified in long-term T cell tumor lines. This cautions against the use of these tumor lines as the exclusive source for RNA and protein in a systematic search for T cell-specific molecules.

Summary

Using a subtractive hybridization procedure we have constructed a cDNA library enriched for sequences present in functional human T cell lines, but not in human EBV-transformed B cell lines. We have isolated a cDNA clone, AH2-519, representing a novel gene, designated 519. This novel gene is expressed in functional human cytolytic and Th cell lines but not in a variety of other cell

lines, including several long-term human T cell tumor lines. The expression of gene 519 is inducible in cultures of normal human PBL using antigenic or mitogenic stimulation. Neither the DNA sequence determined from a full-length cDNA clone overlapping with clone AH2-519 nor the amino acid sequence of its predicted protein product has significant homology to published sequences in the GenBank or NBRF databases. The restricted expression of gene 519 suggests that its gene product is involved in the growth and/or differentiation of normal T cells. The data also show that normal, nontransformed, functional T cells express gene products that can not be readily identified in long-term tumor lines of the same cell lineage.

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