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Immunology

Identification, cloning, and characterization of an immune activation gene

(differential hybridization/immune activation gene/baculovirus/cytokine)

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ABSTRACT **We have identified an immune activation gene, denoted Act-2, by differential hybridization screening of an activated T-cell library. The gene is induced rapidly after T-cell activation with phytohemagglutinin, B-cell activation with *Staphylococcus aureus* Cowan I, and monocyte activation with lipopolysaccharide. We have isolated a cDNA containing the full-length coding region. The deduced amino acid sequence predicts an open reading frame of 92 amino acids, including a very hydrophobic N terminus, which by weight matrix score is predicted to be a signal peptide. Using a baculovirus expression system, we have shown that this gene encodes a secreted product. It is therefore possible that Act-2 represents a newly discovered cytokine.**

T cells play a pivotal role in the regulatory and effector functions of the immune response. Upon stimulation by antigens or mitogenic lectins, a series of biochemical events occurs, including an increase in intracellular calcium, phosphorylation of proteins, and increased inositol phospholipid turnover (1, 2). These events generate signals that lead to the activation of cellular genes and the production of cellular proteins that are not expressed or are very weakly expressed by resting cells. These induced proteins are essential for the proliferation and differentiation of T lymphocytes into effector T cells mediating helper, supressor, or cytotoxic T-cell functions.

We were interested in characterizing genes in T lymphocytes that are induced in response to mitogen stimulation, with the goal of eventually identifying their functional gene products and characterizing their roles in mediating T-cell function and the immune response. One successful approach to identifying genes activated by mitogenic stimuli is to use the technique of differential hybridization (3). The success of this method depends on the finding that although there is extensive overlap between genes expressed in resting and activated cells, each state is characterized by a small number of high abundance mRNAs that encode proteins characteristic of the particular activation state (23).

We report the cDNA cloning, sequencing, and characterization of a gene, denoted Act-2, which was identified by differential screening of an activated human T-cell cDNA library.§

MATERIALS AND METHODS

Cell Culture. Circulating human peripheral blood mononuclear cells (PBMCs) were obtained from healthy volunteers and isolated by Ficoll/Hypaque (LSM, Litton Bionetics) gradient centrifugation. Cells were generally cultured at 1–2 × 10^6 cells per ml overnight in RPMI 1640 medium containing 10% fetal bovine serum (FBS), L-glutamine, and antibiotics. Phytohemagglutinin (PHA; Burroughs Wellcome, Research Triangle Park, NC) and phorbol 12-myristate 13-acetate (PMA; Sigma) were used at 0.5 μg/ml and 50 ng/ml, respectively, unless otherwise indicated. B lymphocytes were purified by incubating PBMCs (4% nonspecific esterase-positive cells) with 2-aminoethylisothiouronium bromide-treated sheep erythrocytes and removing the rosette-focusing cells. In some experiments, monocytes were depleted by plastic adherence. Such cells were 80% surface immunoglobulin-positive latex noningesting cells and were cultured for 0–72 hr in the presence of a 1:10,000 dilution of *Staphylococcus aureus* Cowan I (SAC; Calbiochem–Behring). Monocytes were prepared by elutriation of PBMCs and were 95% pure by Giemsa stain and flow cytometry. Cells were cultured for 8 hr in the presence of lipopolysaccharide (10 μg/ml) (LPS; Sigma).

Cell Lines. T-cell lines (Jurkat, HUT-102B2, Molt-4, CEM, and Hut-78), B-cell lines (Raji, SB, Nall-1, 8392, GM4672, U266, and SUDHL-6), and myeloid lines (K562 and U937) were grown in RPMI 1640 medium containing 10% FBS. Fibroblast 4312 and 4429 and osteosarcoma 5887 cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) containing 15% FBS (4). Human fetal lung fibroblast HFL-1 cells were passaged to a density of 10^6 cells per 175-cm^2 tissue culture flask in 25 ml of DMEM containing 10% FBS and were allowed to grow to confluence over 7 days at 37°C. The cells were stimulated by removing the depleted medium and replacing it with fresh DMEM containing 20% FBS. Early passage HFL-1 cells were used for all experiments.

Preparation of a cDNA Library from Activated Human PBMCs. PBMCs were activated with PHA and PMA as described (5), total cellular RNA was extracted (6), and mRNA was isolated by oligo(dT)-cellulose chromatography. Double-stranded cDNA was prepared by a modification of the procedure of Okayama and Berg (5, 7).

Screening of the cDNA Library. The library was screened in duplicate by the procedure of Grunstein and Hogness (3) with ^{32}P-labeled first-strand cDNA probes derived from induced or uninduced PBMCs. Nitrocellulose filters were incubated for 3 hr at 65°C in 0.1 M NaH_2PO_4, pH 6.8/0.85 M NaCl/1 mM EDTA/10× Denhardt's solution (1× Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone)/0.1% sodium dodecyl sulfate (SDS)/salmon sperm DNA (100 μg/ml)/poly(A) (10 μg/ml) (prehybridization solution). The filters were hybridized at 65°C for 48

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Abbreviations: PBMCs, peripheral blood mononuclear cells; FBS, fetal bovine serum; PHA, phytohemagglutinin; SAC, *Staphylococcus aureus* Cowan I; LPS, lipopolysaccharide.

§The sequence reported in this paper is being deposited in the EMBL/GenBank data base (accession no. J04130).

hr with fresh prehybridization solution containing 10% dextran sulfate and the radioactive probe. The filters were washed four times for 20 min at 65°C in 2× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate)/0.5% SDS and twice for 30 min at 65°C with 0.1× SSC/0.1% SDS and then autoradiographed.

Screening the HUT-102B2 cDNA Library. To identify a full-length cDNA, 10^6 phage plaques from a HUT-102B2 cDNA library, constructed in λgt10 (8), were screened with a partial length Act-2 cDNA insert labeled by the random priming method (9). Phage DNA was bound to nitrocellulose and hybridized overnight at 42°C in 50% formamide/5× Denhardt's solution/5× SSPE (1× SSPE = 0.18 M NaCl/10 mM sodium phosphate, pH 7.4/1 mM EDTA)/0.1% SDS/ salmon sperm DNA (100 μg/ml). Filters were washed three times for 20 min in 2× SSC/0.1% SDS, then twice for 30 min at 56°C in 0.1× SSC/0.1% SDS, and autoradiographed.

DNA Sequencing. Sequencing was performed by the dideoxy chain-termination method using M13 phage DNA and Sequenase (United States Biochemical, Cleveland) according to the manufacturer's recommendations.

Northern Blot Analysis. Either total cellular RNA (10 μg) or poly(A)$^+$ RNA (4–5 μg) was electrophoresed on formaldehyde gels, transferred to nitrocellulose, and hybridized to ^{32}P-labeled Act-2 cDNA.

Genomic Southern Blot. DNA (10 μg) from either U937 or 428 cells was digested with the indicated enzymes, analyzed on a 1% agarose gel, and transferred to GeneScreen*Plus* nylon membranes (DuPont) and hybridized according to the manufacturer's suggested protocol.

***In Vitro* Transcription and Cell-Free Translation.** The 0.7-kilobase (kb) Act-2 cDNA was digested with *Ava* I, filled in with Klenow and dNTPs, and then digested with *Eco*RI to liberate a nearly full-length fragment (base 27 through the 3′ end of the insert) from which the artificial 5′ GC tail had been removed. This fragment was subcloned into *Sma* I/*Eco*RI-digested pGEM-1 (Promega Biotec, Madison, WI). RNA was transcribed from both strands by using SP6 or T7 promoters, as per instructions from Promega Biotec. Reactions were terminated by digesting the DNA template with RNase-free DNase I, and the RNA was purified by phenol/chloroform extraction and ethanol precipitation. RNA was translated *in vitro* with rabbit reticulocyte and wheat germ lysates (Promega Biotec or Amersham) by using [^{35}S]cysteine (>600 Ci/mmol; 1 Ci = 37 GBq; Amersham). Some translations were performed in the presence of canine pancreatic microsomal membranes. Translation products were analyzed on 15% SDS/polyacrylamide gels.

Expression of the Act-2 cDNA. The 0.7-kb Act-2 cDNA was cloned in the *Bam*HI site of the baculovirus polyhedrin plasmid pAc373 (10) to generate pAc-Act2, thereby placing the Act-2 cDNA under control of the polyhedrin promoter. SF9 cells were cotransfected with pAc-Act2 and wild-type AcNPV strain E_2 DNA. A recombined baculovirus, vAc-Act2, was isolated and purified by a procedure of successive rounds of plaque hybridization (10). When plaque-purified recombinant virus was obtained, SF9 cells were infected and biosynthetically labeled with [^{35}S]cysteine or [^{35}S]methionine. Radiolabeled Act-2 protein, which was secreted into the cell supernatant, was isolated on SDS gels, eluted in water, and subjected to sequential Edman degradation on a protein sequencer (Applied Biosystems model 477) using the manufacturer's ATZ program. Fractions were assayed by liquid scintillation counting.

RESULTS

Identification of an Act-2 cDNA. Ten thousand recombinant bacterial clones from the activated PBMC cDNA library were screened in duplicate using first-strand cDNA probes derived from RNA from activated or resting PBMCs. Act-2 was a 0.4-kb cDNA clone that hybridized selectively and strongly only to the induced probe. This finding was confirmed by a Northern blot of RNA from unstimulated PBMCs, PBMCs activated for 16 hr with PMA or PHA, and HUT-102B2 cells, a human T cell lymphotropic virus type I (HTLV-I)-transformed T-cell line (Fig. 1*A*). No significant signal was detected in resting PBMCs, but a band of ≈0.9 kb was detected in activated PBMCs and HUT-102B2 cells.

Cell Specificity. To determine the range of cell types expressing the Act-2 gene, we examined a variety of T-cell, B-cell, and nonlymphoid cell lines by Northern blot analysis. Act-2 was expressed in HUT-102B2 cells (Fig. 1*A*), but not in Molt-4 or CEM T cells (data not shown). Act-2-specific mRNA was similarly not detected in HeLa or K562 cells in the simian virus 40-transformed fibroblast cell lines 4312 and 4429, or in the osteosarcoma cell line 5887. Although Act-2 mRNA was minimally detectable in the Epstein–Barr virus-infected B-cell lines Raji, GM4672, SB, and 8392, its expression was not detected in Nall-1 (pre-B cells), SUDHL-6, or in plasmacytoma cell lines U266 or 8662 (data not shown). Interestingly, Jurkat T cells when induced with both PHA and PMA (Fig. 1*B*), but not with either agent separately, expressed significant amounts of Act-2, as did PMA-stimulated U937 cells (derived from a human histiocytic lymphoma but retaining monocyte-like characteristics; Fig. 1*C*).

To further characterize the temporal expression of Act-2 mRNA in lymphocyte activation, a time course of Act-2 mRNA expression was performed in freshly isolated PBMCs activated with PHA (Fig. 2*A*). Act-2 mRNA was expressed at low or undetectable levels in resting PBMCs, but it increased rapidly and dramatically in response to mitogen stimulation. Peak levels occurred at ≈4 hr and then declined to negligible levels by 24 hr. Thus, Act-2 expression is both rapidly induced and terminated in response to PHA.

An analogous examination of B cells stimulated with SAC (Fig. 2*B*) also indicated a rapid induction and disappearance of Act-2 mRNA. We also demonstrated that monocytes stimulated for 8 hr with LPS expressed Act-2, whereas resting monocytes did not (Fig. 2*C*). The observation that the Act-2 gene was expressed in several different normal cell types exposed to mitogenic stimuli suggested that Act-2 expression might be induced in all normal cells after activation to traverse the cell cycle. In view of parallels made between the proliferative response of resting lymphocytes to

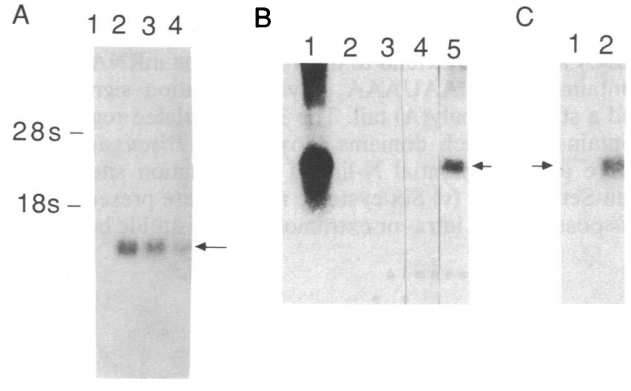

FIG. 1. Northern blots using ^{32}P-labeled Act-2 cDNA probe and RNA from a variety of human cell types. (*A*) Total cellular RNA (10 μg) from PBMCs not stimulated (lane 1), or stimulated with PMA (lane 2), or PHA (lane 3), and poly(A)$^+$ RNA (4 μg) from HUT-102B2 cells (lane 4). (*B*) Total cellular RNA (10 μg) from PBMCs stimulated for 1 hr with PMA plus PHA (lane 1) and from Jurkat T cells not stimulated (lane 2), or stimulated for 4 hr with PHA (lane 3), PMA (lane 4), or PHA plus PMA (lane 5). (*C*) Total cellular RNA (10 μg) from U937 cells not stimulated (lane 1) or stimulated for 15 min with PMA (lane 2).

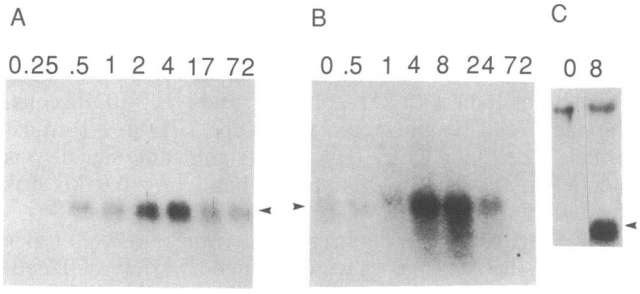

FIG. 2. Northern blots using 10 μg of total cellular RNA. (*A*) RNA prepared from unstimulated PBMCs or PBMCs stimulated with PHA for 0.25, 0.5, 1, 2, 4, 17, or 72 hr. (*B*) RNA prepared from unstimulated B cells or B cells stimulated with SAC for 0.5, 1, 4, 8, 24, or 72 hr. (*C*) RNA from unstimulated monocytes or monocytes stimulated for 8 hr with LPS.

mitogens and of quiescent fibroblasts to growth factors regarding c-*myc* expression (11), we examined Act-2 expression in quiescent and serum-stimulated primary cultures of normal human fetal lung (HFL-1) fibroblasts. In contrast to c-*myc*, however, we were unable to induce expression of Act-2 mRNA (data not shown).

Identification and Sequencing of a Full-Length Act-2 cDNA. Because of disparity between the sizes of the cDNA and the band detected on Northern blots, we proceeded to isolate a longer cDNA. Using the 0.4-kb Act-2 cDNA insert as a probe, we screened 10^6 λgt10 plaques from an amplified HUT-102B2 cDNA library (8). Four clones with identically sized inserts of ≈0.7 kb were identified, all of which hybridized to the same 0.9-kb mRNA identified by the 0.4-kb cDNA. The DNA and deduced amino acid sequences are shown in Fig. 3. The salient features of the sequences can be summarized as follows: (*i*) Act-2 represents a newly discovered gene. Comparison of the DNA sequence and the predicted amino acid sequence by DNA and protein homology search programs did not reveal any homology with published sequences in GenBank (version 55, March 31, 1988) and National Biomedical Research Foundation (versions 16 and 34, March 31, 1988) data bases. (*ii*) The DNA sequence contains an open reading frame of 276 base pairs, corresponding to 92 amino acids. This reading frame utilizes the most 5′ AUG, which generally is the one utilized in eukaryotic translation initiation. Furthermore, the region of the first AUG has good homology with the consensus sequence determined for eukaryotic start sites (12). (*iii*) The cDNA appears to extend to the 3′ end of the mRNA since it contains classic AAUAAA polyadenylylation signals (13) and a start of a poly(A) tail. The 3′ untranslated region also contains A+T-rich domains (boxed, see *Discussion*). (*iv*) There are no potential N-linked glycosylation sites (Asn-Xaa-Ser or Thr). (*v*) Six cysteine residues are present; thus, it is possible that intra- or extramolecular disulfide bonds are

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TTCCCCCCCCCCCCCCCCCCCCGCCCGAGCACAGGACACAGCTGGGTTCTGAAGCTTC      60

TGAGTTCTGCAGCCTCACCTCTGAGAAAACCTCTTTTCCACCAATACCATGAAGCTCTGC    120
                                                  MetLysLeuCys
                                                             1

GTGACTGTCCTGTCTCTCCTCATGCTAGTAGCTGCCTTCTGCTCTCCAGCGCTCTCAGCA    180
ValThrValLeuSerLeuLeuMetLeuValAlaAlaPheCysSerProAlaLeuSerAla
                  10                  20

CCAATGGGCTCAGACCCTCCCACCGCCTGCTGCTTTTCTTACACCGCGAGGAAGCTTCCT    240
ProMetGlySerAspProProThrAlaCysCysPheSerTyrThrAlaArgLysLeuPro
                  30                  40

CGCAACTTTGTGGTAGATTACTATGAGACCAGCAGCCTCTGCTCCCAGCCAGCTGTGGTA    300
ArgAsnPheValValAspTyrTyrGluThrSerSerLeuCysSerGlnProAlaValVal
                  50                  60

TTCCAAACCAAAAGAAGCAAGCAAGTCTGTGCTGATCCCAGTGAATCCTGGGTCCAGGAG    360
PheGlnThrLysArgSerLysGlnValCysAlaAspProSerGluSerTrpValGlnGlu
                  70                  80

TACGTGTATGACCTGGAACTGAACTGAGCTGCTCAGAGACAGGAAGTCTTCAGGGAAGGT    420
TyrValTyrAspLeuGluLeuAsnEnd
                  90

CACCTGAGCCCGGATGCTTCTCCATGAGACACATCTCCTCCATACTCAGGACTCCTCTCC    480

GCAGTTCCTGTCCCTTCTCTTAATTTAATCTTTTTTATGTGCCGTGTTATTGTATTAGGT    540
                    *****

GTCATTTCCATTATTTATATTAGTTTAGCCAAAGGATAAGTGTCCTATGGGGATGGTCCA    600
          ********

CTGTCACTGTTTCTCTGCTGTTGCAAATACATGGATAACACATTTGATTCTGTGTGTTTT    660

CCATAATAAAACTTTAAAATAAAATGCAGACAGTTAAAAAAAAAAAAAAAAA            711
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FIG. 3. DNA and deduced amino acid sequences for the Act-2 cDNA. Both cDNA strands were sequenced. Nucleotides are numbered at the right of each line. Every 10th amino acid is numbered. Amino acid 1 corresponds to the translation start site. The signal peptide (italics) is cleaved after Ser-23, so that Ala-24 is the first amino acid of the mature protein. The initial bases in the DNA sequence represent part of *Eco*RI linker and an artificial C tail added during preparation of the cDNA. In one sequencing reaction, the underlined bases 584–592 sequenced as CCCCTATGG for unclear reasons. Polyadenylylation consensus sequences are boxed. The bases marked by asterisks are perfect matches with the consensus sequences referred to in the text.

formed. (*vi*) Examination of a Kyte–Doolittle hydrophobicity plot (Fig. 4) generated from the deduced amino acid sequence reveals an extremely hydrophobic N terminus. Using the von Heijne weight matrix analysis, this appears to be a signal peptide (calculated score of 11.3), with cleavage predicted between Ser-23 and Ala-24 (14), a cleavage site also used in the cytokines interleukin 2 (15), interleukin 3 (16), and granulocyte–macrophage colony-stimulating factor (17).

To demonstrate that Act-2 encodes a functional mRNA, we subcloned the cDNA insert into pGEM-1 and transcribed both sense and antisense strands by using the SP6 and T7 promoters. The resulting RNAs were translated in wheat germ and reticulocyte lysates (Fig. 5). The sense (lane B) but not the antisense (lane A) strand resulted in a detectable product, which migrated with an apparent mass of 11,000–13,000 Da, similar to the calculated molecular mass of 10,199 Da. Unfortunately, an artifact that comigrated with the Act-2 primary translation product was seen on gels with the reticulocyte lysate system; the wheat germ lysate system thus gave cleaner results. To evaluate cleavage of the putative

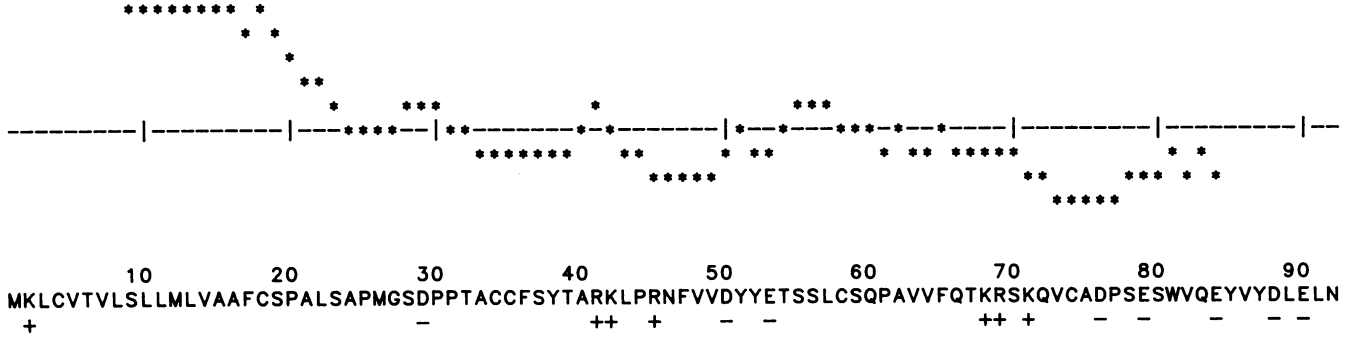

FIG. 4. Kyte–Doolittle hydrophobicity plot (window size, 17) of the Act-2 amino acid sequence (single-letter code).

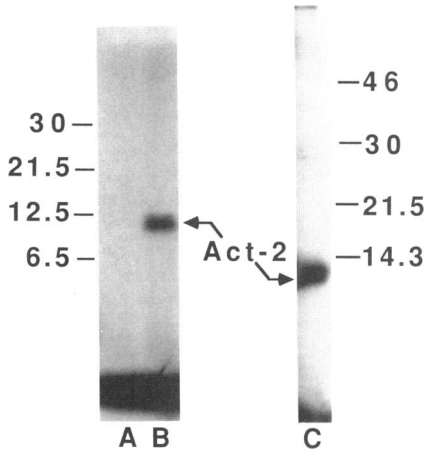

FIG. 5. Translation of antisense (lane A) and sense (lane B) Act-2 mRNA in a wheat germ lysate cell-free translation system using [^{35}S]cysteine. Lane C, canine microsomal membranes were cotranslationally added to a rabbit reticulocyte cell-free translation system using Act-2 sense mRNA, followed by centrifugation at 15,000 × *g*, washing the pellet in sucrose/Tris/NaCl, and analyzing the membrane fraction on a SDS gel. The primary translation product was associated with the membrane fraction.

signal peptide and translocation into the lumen of the endoplasmic reticulum, we next performed experiments with canine microsome membranes. As microsomal membranes are often more efficient in the reticulocyte lysate system, we performed cotranslational translocation/cleavage experiments with a reticulocyte lysate system. Membranes were pelleted at 31,000 × *g*, washed in 10 mM Tris·HCl/0.15 M NaCl/0.2 M sucrose, pH 7.5, and then boiled in sample buffer and analyzed on SDS gels. The primary translation product, now free of the artifact, was associated with the membrane fraction (lane C), suggesting that the N terminus functions like a signal peptide. Based on the sequence, we expected a change of 2391 Da if the putative signal peptide were cleaved; however, no significant change was noted, suggesting that the putative signal peptide might not be cleaved. Treatment of the membrane-associated band in lane C with proteinase K in the presence or absence of Triton X-100 resulted in much more complete degradation in the presence of detergent (data not shown). These data indicate that the protein associates with membranes, but cleavage of the signal peptide could not be demonstrated.

Table 1. N-terminal sequencing of Act-2 protein labeled with [^{35}S]methionine or [^{35}S]cysteine

Cycle	Exp. 1 [^{35}S]Methionine, cpm	Exp. 2 [^{35}S]Cysteine, cpm
1	486	80
2	563	77
3	10,199	76
4	3,170	123
5	1,002	201
6		360
7		389
8		432
9		406
10		478
11		2766
12		4651
13		1986
14		916

The high carryover [in cycle 4 from the methionine at cycle 3 (see Exp. 1) and in cycles 13 and 14 from the cysteines at positions 11 and 12 (see Exp. 2)] were expected due to prolines at positions 2, 7, and 8 of the mature protein (see Fig. 4), which are inefficiently cleaved.

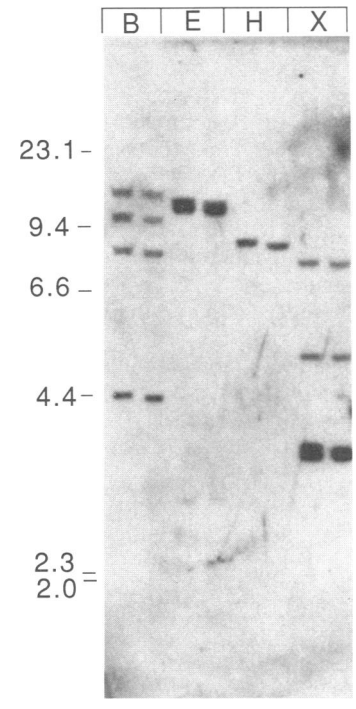

FIG. 6. Genomic Southern blot using DNA from 428 cells (lanes 1, 3, 5, and 7) or U937 cells (lanes 2, 4, 6, and 8) digested with *Bam*HI (B, lanes 1 and 2), *Eco*RI (E, lanes 3 and 4), *Hin*dIII (H, lanes 5 and 6), or *Xba* I (X, lanes 7 and 8), and hybridized to the full-length Act-2 cDNA insert.

To determine that Act-2 encodes a secreted product and that the signal peptide is cleaved, we expressed Act-2 as a recombinant baculovirus in SF9 cells. Cells were biosynthetically labeled with [^{35}S]methionine or [^{35}S]cysteine, and the supernatant was recovered and subjected to electrophoresis on a SDS gel. For each label, the specific Act-2 protein band was detected, eluted from the gels, and sequenced. The detection of a methionine at cycle 3 and cysteines at cycles

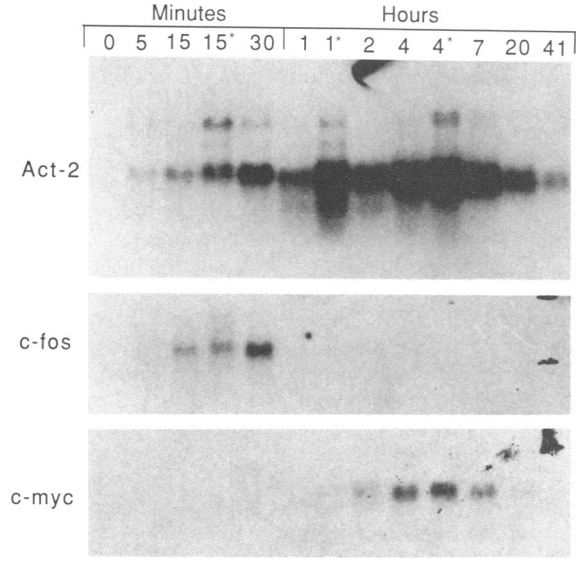

FIG. 7. Northern blot using 10 μg of total cellular RNA. Expression of Act-2 (0.9 kb), c-*fos* (2.2 kb), and c-*myc* (2.4 kb) in PBMCs stimulated with PHA or PHA plus PMA (asterisks). The same blot was sequentially hybridized with each probe and stripped between hybridizations.

11 and 12 (Table 1) confirm that the signal peptide is cleaved between Ser-23 and Ala-24 of the primary translation product (see Fig. 3), as predicted.

Next, we used the cDNA to hybridize to a genomic Southern blot (Fig. 6). A comparatively simple pattern of digestion was obtained. The same blot was hybridized with an interleukin 2 receptor cDNA [the interleukin 2 receptor is encoded by a single copy gene (18)], and bands of similar intensity were identified (data not shown). These data are most consistent with Act-2 representing a single copy or low copy number gene.

Finally, we directly compared the time course of expression of Act-2, c-*fos*, and c-*myc* in PBMCs stimulated with PHA or PHA plus PMA, and we found that Act-2 mRNA is induced coordinately with c-*fos* and its peak expression coincides with that of c-*myc* (Fig. 7).

DISCUSSION

In this study, we identified and characterized a human activation gene, denoted Act-2 (activated PBMC cDNA, number 2), in a cDNA library prepared from mRNA from normal activated PBMCs. Act-2 encodes an open reading frame of 92 amino acids. It contains a very hydrophobic N terminus that was strongly predicted to be a signal peptide. Although by *in vitro* translation analyses we could not demonstrate cleavage of the signal peptide, we have expressed a recombinant Act-2/baculovirus vector in SF9 insect cells and found that Act-2 protein is abundantly secreted into the supernatant. Moreover, N-terminal sequencing of radiolabeled protein shows that the signal peptide is cleaved as predicted.

The 3′ untranslated region is of significant interest in that it is A+T-rich and contains the consensus sequences ATTTA (19) and TTATTTAT (20) that have been identified as common sequences in a variety of protooncogenes and secreted factors, including tumor necrosis factor, lymphotoxin, interleukin 1 (both α and β), multiple interferons, and granulocyte–macrophage colony-stimulating factor. The TTATTTAT consensus is not commonly found in mammalian mRNAs in general but is prevalent in mRNAs encoding proteins related to the inflammatory response (20); thus, its presence in Act-2 is supportive of the idea that this gene may represent a newly discovered cytokine. These sequences also correlate with relative instability of mRNA (19), consistent with the rapid decline from peak levels of induced mRNA.

This gene is especially interesting in view of its rapid time course of activation. Human resting PBMCs represent normal cells in a physiological quiescent state (G_0) (21, 22). Upon activation with antigen or mitogen, there is cellular enlargement, increase in RNA content, transcription of new genes, and synthesis of new proteins. The cells are thereby rendered receptive to further signals, such as interleukin 2, that promote cellular proliferation. The induction of Act-2 expression in response to mitogenic stimuli and its coordinate expression with c-*fos* and c-*myc* are consistent with the possibility that Act-2 may represent an early and potentially important factor in cell growth and proliferation.

Act-2 was minimally or not expressed in resting PBMCs. However, it was rapidly induced after activation of T cells with PHA or PMA, B cells with SAC, or monocytes with LPS. However, we know that Act-2 is not expressed in every actively growing cell, as evidenced by its nonexpression in HeLa and K562 cells and its failure to be induced in response to serum stimulation of quiescent human fibroblasts. When anti-Act-2 antibodies and larger quantities of purified Act-2 protein are available, it should be possible to further elucidate the potential biologic roles of the Act-2 protein in the immune response.

Note Added in Proof. Recently, another group has identified a cDNA, denoted PAT744, which also corresponds to the Act-2 gene (Uli Siebenlist and Kathleen Kelly, personal communication).

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