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Molecular Cloning, Sequence Analysis, and Functional Expression of a Novel Growth Regulator, Oncostatin M

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Oncostatin M is a polypeptide of M_r ~28,000 that acts as a growth regulator for many cultured mammalian cells. We report the cDNA and genomic cloning, sequence analysis, and functional expression in heterologous cells of oncostatin M. cDNA clones were isolated from mRNA of U937 cells that had been induced to differentiate into macrophagelike cells by treatment with phorbol 12-myristate 13-acetate, and a genomic clone was also isolated from human brain DNA. Sequence analysis of these clones established the 1,814-base-pair cDNA sequence as well as exon boundaries. This sequence predicted that oncostatin M is synthesized as a 252-amino-acid polypeptide, with a 25-residue hydrophobic sequence resembling a signal peptide at the N terminus. The predicted oncostatin M amino acid sequence shared no homology with other known proteins, but the sequence of the 3′ noncoding region of the cDNA contained an A+T-rich stretch with sequence motifs found in the 3′ untranslated regions of many cytokine and lymphokine cDNAs. Oncostatin M mRNA of ~2 kilobase pairs was detected in phorbol 12-myristate 13-acetate-treated U937 cells and in activated human T cells. Transfection of cDNA encoding the oncostatin M precursor into COS cells resulted in the secretion of proteins with the structural and functional properties of oncostatin M. The unique amino acid sequence, expression by lymphoid cells, and growth-regulatory activities of oncostatin M suggest that it is a novel cytokine.

Cells of the immune system secrete numerous cytokines and lymphokines which function in a variety of physiological processes, including regulation of immune responses and wound healing (12; reviewed in references 13 and 24). They also may play a role in pathological conditions such as chronic inflammatory disorders (9). Because of their potential medical importance, much effort has gone into the identification and characterization of new cytokines.

Oncostatin M is a heat- and acid-stable growth regulator of M_r ~28,000 that was isolated from serum-free supernatants of U937 histiocytic leukemia cells that had been induced to differentiate into macrophagelike cells by treatment with phorbol 12-myristate 13-acetate (PMA) (40). Oncostatin M was originally identified by its ability to inhibit the replication of A375 melanoma and other human tumor cells but not normal human fibroblasts. The biological properties and N-terminal sequence of purified oncostatin M suggested that oncostatin M was a unique cell growth regulator. This factor was also purified from the culture medium of activated human T lymphocytes and shown to act synergistically with transforming growth factor β1, but not gamma interferon, in inhibiting proliferation of A375 melanoma cells (5). High-affinity binding sites for oncostatin M have been identified in cell lines whose growth is regulated by this factor (20a).

To facilitate further studies on the properties, biological activities, and potential therapeutic applications of oncostatin M, we have undertaken the molecular cloning and expression of this growth regulator. Here we report the isolation of cDNA and genomic clones for oncostatin M, sequence analysis of these clones, and expression of a functional oncostatin M gene product in mammalian cells.

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MATERIALS AND METHODS

Cell culture. U937 and A375 cells were cultured as described previously (40). COS cells (15), obtained from Brian Seed, Massachusetts General Hospital, Boston, were routinely maintained in Dulbecco modified Eagle medium containing 10% fetal bovine serum, 100 μg of streptomycin per ml, and 100 U of penicillin per ml. Phytohemagglutinin-activated peripheral blood T lymphocytes, prepared as previously described (5), were generously provided by T. Joseph Brown.

Isolation of RNA and DNA. U937 cells were induced with PMA at 10 ng/ml; at 16, 36, and 52 h after induction, RNA was isolated (8) from equivalent numbers of adherent cells. RNA was isolated from T lymphocytes 72 h after activation with phytohemagglutinin as described previously (5). Poly(A) RNA fractions were purified by affinity chromatography on oligo(dT) cellulose (3). High-molecular-weight DNA was isolated from human brain tissue (22).

Construction and screening of cDNA libraries. Poly(A) RNA from PMA-treated U937 cells was pooled and used as a template for oligo(dT)-primed cDNA synthesis. Additional 5′ clones were subsequently obtained by priming cDNA synthesis with an oligonucleotide (5′-CAGGAGTCTGCTG GTGTCCTGCA-3′, the complement of nucleotides 146 to 168; Fig. 1) derived from the oncostatin M cDNA clone pOM46. The cDNA was dG tailed and cloned into *Eco*RI-digested lambda gt10 by using a linker oligonucleotide, 5′-AATTCCCCCCCCCCCC-3′ (29).

The library primed with oligo(dT) was screened with a 50-mer oligonucleotide derived from the amino acid sequence of the Lys-C peptide K4b (Table 1) of oncostatin M (5′-TTCTCCAGGTCCTCAATGTTCAGGCCAGACCGCT CCAGGTCCTGGGCCTT-3′), which had been end labeled with ^{32}P by using polynucleotide kinase. The codons were chosen to reflect the most frequently occurring codon for each amino acid in humans (19). Further screening of posi-

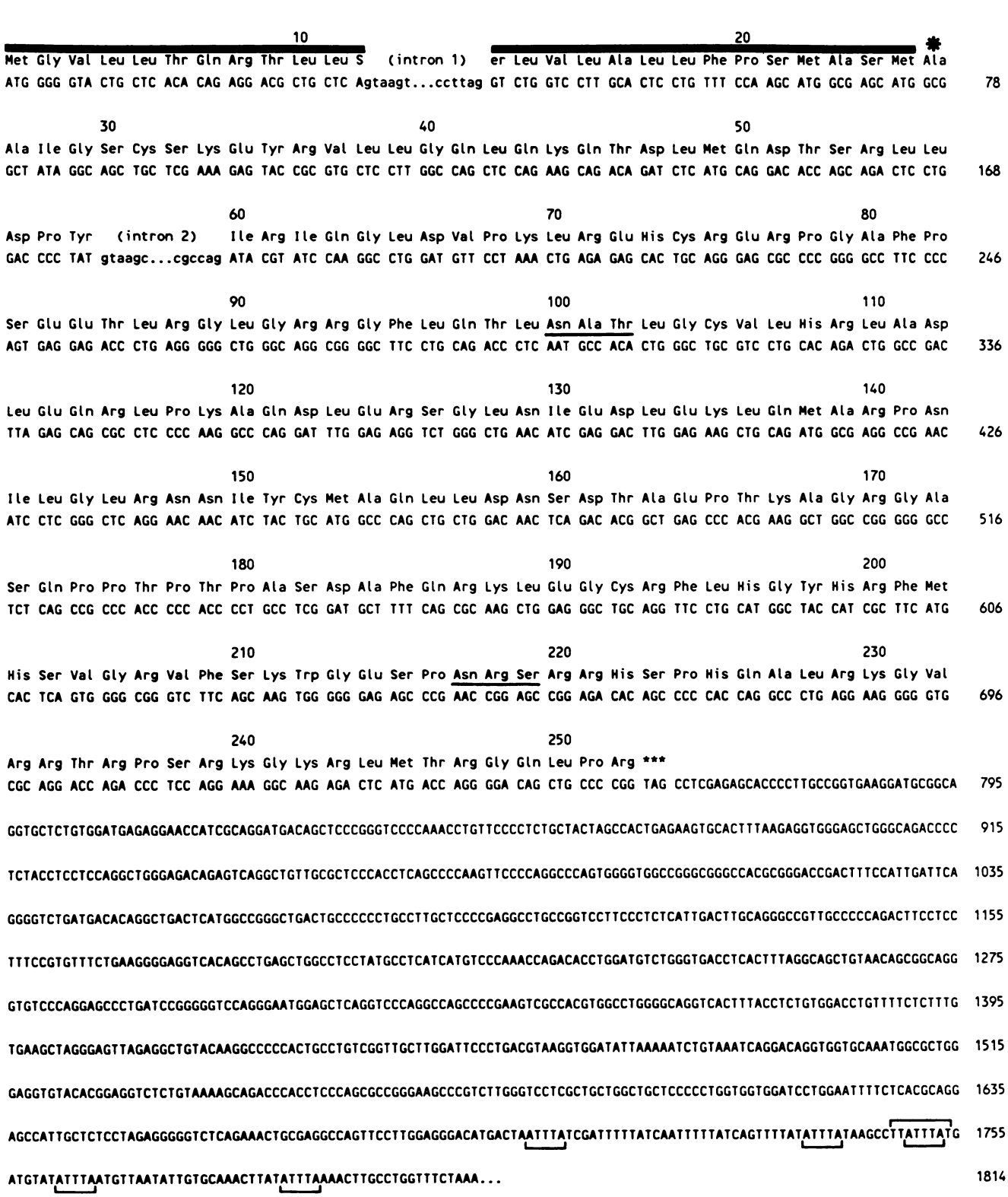

FIG. 1. Nucleotide and deduced protein sequences for the consensus oncostatin M cDNA showing consensus nucleotide sequence of oncostatin M cDNA (uppercase), intron junction sequences (lowercase), and deduced protein sequence. Indicated are potential N-linked glycosylation sites (underlined), the signal peptide (═), the N terminus of mature oncostatin M (*), and sequence motifs commonly found in 3′ noncoding regions of cytokine and lymphokine mRNAs (35) (⌊_⌋), and inflammatory mediator mRNAs (6) (⌐¬).

TABLE 1. Amino acid sequences from oncostatin M

Peptide	Position[a]	Yield[b] (pmol)	Sequence[c]
K6	45–60	4	QTDLMQDTS(T)LL(T)PYI
K8	70–83	7	L(R)E(H)CRERPGAFP(S)E
K4b	120–135	8	AQDLERSGLNIEDLEK
K5	168–176	3	AGRGASQP(P)
K2	212–219	10	X(G)ESPNRS

[a] Numbers refer to amino acids in the precursor sequence (Fig. 1).
[b] Determined by sequence analysis.
[c] Sequences of Lys-C fragments of oncostatin M purified from PMA-treated U937 cells. Residues in parentheses indicate tentative assignments; X, unidentified residue.

tive clones was done on Southern blots of cDNA inserts by using a 41-mer oligonucleotide (5′-ATGTATGGGGTCAGC AGGGTGGAGGTGTCCTGCATCAGGTC-3′) derived from amino acids 3 to 16 of the Lys-C peptide K6 (Table 1). The library primed with the 5′ oligonucleotide was screened with a 376-base-pair (bp) *Pvu*II fragment from the 5′ end of the oncostatin M cDNA clone pOM46 (Fig. 2).

Construction and screening of genomic libraries. High-molecular-weight DNA from human brain tissue was digested with *Hin*dIII and fractionated by electrophoresis in a 1% agarose gel; fragments in the size range of 9 to 12 kbp were isolated and cloned into lambda L47.1 at the *Hin*dIII site. Nitrocellulose plaque lifts were probed with the ^{32}P-labeled (28) *Pvu*II fragment from the 3′ end of the coding region of the cDNA clone, pOM46 (Fig. 2).

DNA sequence analysis. cDNAs that hybridized specifically to the probes were excised from the lambda gt10 vector, subcloned into the *Eco*RI site of the plasmid vector pEMBL18+ (11), and sequenced on both strands by the dideoxy-chain termination method (30). Sequencing was performed on various restriction fragments and exonuclease III deletion fragments (16) and by specific priming with synthetic oligonucleotides. Sequences were analyzed by using GenePro software (Riverside Scientific, Seattle, Wash.).

RNA analysis. RNA samples were fractionated by electrophoresis in 1% agarose-formaldehyde gels, transferred to nylon membranes (Hybond; Amersham Corp., Arlington Heights, Ill.), and hybridized with the ^{32}P-labeled 1.5-kbp *Bgl*II-*Eco*RI fragment from the 3′ end of the oncostatin M cDNA sequence (Fig. 2). Hybridization was carried out in 50% formamide–5× SSPE (SSPE is 0.15 M NaCl, 0.2 M NaH_2PO_4 [pH 7.0], and 0.02 M EDTA)–5× Denhardt solution–10% dextran sulfate–20 μg of denatured salmon sperm DNA per ml (22). Filters were washed at 65°C in 0.1× SSC (SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and autoradiographed.

Expression of oncostatin M in COS cells. A *Hin*PI-*Bgl*II fragment containing oncostatin M signal peptide was isolated from plasmid pOM25. The remaining coding sequence was isolated as a *Bgl*II-*Xho*I fragment from plasmid pOM46. These fragments were inserted into the πH3MPy vector (πH3M [2] modified by the addition of the polyomavirus origin of replication [I. Stamenkovic, E. A. Clark, and B. Seed, EMBO J., in press]). This construct (pSPOM) contained the coding region of the oncostatin M cDNA sequence (Fig. 1), which was confirmed by DNA sequence analysis. pSPOM was transfected into COS cells (37; A. Aruffo and B. Seed, personal communication). After transfection, serum-free culture medium was collected and assayed directly for growth-inhibitory activity or dialyzed against 1 N acetic acid before immunoblot analysis or purification. Cell monolayers were solubilized directly in electrophoresis sample buffer before analysis.

Growth inhibition assay. Culture medium in various concentrations from transfected COS cells was compared with control medium for the ability to inhibit proliferation of A375 cells by using a dye-binding assay (36; Linsley et al., in press). Cells were seeded at 3×10^3 to 4×10^3 cells per well in 96-well plates and incubated with the test solutions in a final volume of 200 μl; proliferation was measured by crystal violet staining after 72 h at 37°C. Under these conditions, addition of 10 ng of oncostatin M purified from culture medium of PMA-treated U937 cells resulted in 50% inhibition of the proliferation of A375 cells.

Electrophoresis and immunoblotting. Samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on gradient acrylamide gels run under reducing conditions. Gels were stained with silver reagent (Bio-Rad Laboratories, Richmond, Calif.) or processed for immunoblot analysis.

For immunoblot analysis, proteins were transferred to nitrocellulose, and the immobilized proteins were stained with a site-specific rabbit antiserum essentially as described previously (21) except that transfer to nitrocellulose was done with a semidry transfer apparatus (Sartorius) and detection of bound antibody was achieved colorimetrically (20), using alkaline phosphatase-conjugated protein A. The antiserum used was prepared against a peptide corresponding to residues 6 to 19 of the amino terminus of oncostatin M (residues 31 to 44 of the precursor sequence shown in Fig. 1).

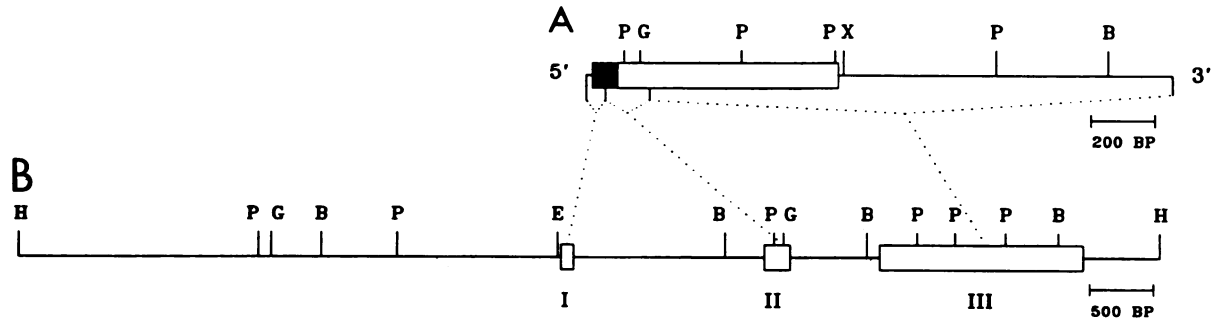

FIG. 2. Molecular maps of human oncostatin M cDNA and gene. (A) Restriction map of the 1,839-bp consensus cDNA, presented 5′ to 3′. The coding region is boxed; the position of the signal sequence is shaded. (B) Map of the 9-kbp *Hin*dIII genomic fragment with the corresponding exons; the three known exons (I, II, and III) are boxed. The extents of the 5′ and 3′ exons have not been completely determined. Restriction enzyme sites: B, *Bam*HI; G, *Bgl*II; E, *Eco*RI; P, *Pvu*II; X, *Xho*I.

Synthesis of peptides, conjugation to bovine pooled immunoglobulin carrier protein, and immunization of rabbits were performed as described by Gentry et al. (14).

Protein purification and peptide sequencing. Oncostatin M was purified from supernatants of U937 cells induced with PMA (40). Purified oncostatin M was reduced with dithiothreitol and S-carboxamidomethylated with iodoacetamide. S-carboxamidomethylated oncostatin M (30 pmol) was cleaved with endoproteinase Lys-C, and peptides were purified and sequenced (23). Recombinant oncostatin M was purified from supernatants of COS cells transfected with the oncostatin M expression construct pSPOM essentially as described elsewhere (20a) except that two sequential size fractionations on a TSK 3000SW column (run in 40% acetonitrile, 0.1% trifluoroacetic acid; LKB Instruments, Inc., Rockville, Md.) were substituted for fractionation on a Bio-Gel P60 column.

RESULTS

Isolation of cDNA and genomic clones. Proteolytic fragments of oncostatin M from U937 cells were prepared and sequenced (Table 1). These sequences were used to derive DNA hybridization probes (19) for screening lambda cDNA libraries made from pooled poly(A) RNA isolated from U937 cells treated with PMA for 16, 36, and 52 h. The cDNA libraries were first screened with a 50-mer oligonucleotide probe corresponding to the amino acid sequence of peptide K4b (Table 1). Eight clones that hybridized specifically with the 50-mer were isolated, purified, and further probed with a 41-mer oligonucleotide corresponding to amino acids 3 to 16 of peptide K6. One of these clones, containing a 2.1-kbp cDNA insert, also hybridized with the 41-mer probe. The insert from this bacteriophage was cloned into the *Eco*RI site of pEMBL18+ (11) and designated pOM46.

The complete nucleotide sequence of the insert cDNA of pOM46 was determined. The cDNA contained a long open reading frame that encoded the amino acid sequence determined for the N terminus of oncostatin M (40) as well as sequences corresponding to five peptide fragments of oncostatin M (Table 1). The amino acid sequence of peptide K6 that was used to derive the 41-mer differed from that predicted by the cDNA clone at two tentatively assigned positions (Table 1).

An additional cDNA library was prepared by using synthetic oligonucleotides derived from the 5′ end of the coding region to prime for cDNA synthesis (see Materials and Methods). The cDNA was cloned into lambda gt10, and one lambda clone containing a 233-bp cDNA insert was isolated that hybridized to a nick-translated ^{32}P-labeled *Pvu*II cDNA fragment (Fig. 2) derived from the 5′ coding region of pOM46. The cDNA insert was cloned into pEMBL18+ (pOM25), and the complete nucleotide sequence was obtained. Comparison of the sequences of pOM46 and pOM25 revealed a 121-bp overlap extending from nucleotide 156 to nucleotide 48 (Fig. 1). Upstream, the sequences completely diverged; pOM25 encoded a hydrophobic region, whereas pOM46 contained a termination codon.

To resolve the discrepancy between the 5′ ends of the cDNA clones, an oncostatin M genomic clone was isolated. The *Pvu*II fragment from the 5′ portion of the coding region (Fig. 2) from pOM46 was labeled with ^{32}P and used to probe a Southern blot of human brain DNA digested with *Hin*dIII, *Bgl*II, *Bam*HI, *Stu*I, *Nco*I, *Xba*I, *Sph*I, and *Eco*RI (data not shown), and a genomic map was determined for the oncostatin M gene (Fig. 2). A 9-kbp *Hin*dIII fragment was selected for cloning, and a genomic library was constructed in bacteriophage lambda L47.1 with size-fractionated human brain DNA. The library was screened with the two *Pvu*II fragments of the cDNA coding region, and one clone that reacted with both of the probes was identified. The 9-kbp genomic fragment was subcloned into pEMBL18+ and designated pOMH9. The DNA sequence of the entire coding portion of the oncostatin M gene and sequences ~500 bp upstream and ~1.5 kbp downstream were obtained. The genomic sequences were compared with those sequences of the oncostatin M cDNA clones. The exon sequences confirmed the sequences of the 233-bp cDNA clone (pOM25) and those of the 3′ 1,770 bp of the cDNA clone (pOM46). The sequences in the remaining 5′ end of pOM46 that differed from those in the 5′ end of pOM25 were not detected in the genomic clone, which suggests that they were an artifact of the initial cDNA library preparation.

Oncostatin M cDNA and genomic sequence. Maps of the genomic and cDNA structures of oncostatin M as determined from the two cDNA clones and the genomic clone are shown in Fig. 2. A consensus cDNA sequence with exon boundaries is shown in Fig. 1. An open reading frame of 784 nucleotides was found to extend from the beginning of the cDNA sequence. Sequences flanking the first ATG (nucleotide 1; Fig. 1) in the reading frame conformed to the consensus sequence for the initiating methionine (17). A total of 25 nucleotides of 5′ noncoding and 1,055 nucleotides of 3′ noncoding sequences were obtained in the two cDNA clones. No poly(A) tract was detected in the cDNA clones, and no consensus polyadenylation signal (AATAAA) was detected in either the 3′ noncoding cDNA sequence or the subsequent 500-bp sequence present in the genomic clone. Thus, either the genomic clone does not contain the 3′ terminus of the mRNA or a sequence different from the AATAAA consensus establishes the end of the mRNA.

The oncostatin M cDNA was found to encode a 252-amino-acid precursor that contains a 25-amino-acid hydrophobic region resembling a signal sequence at the N terminus (Fig. 1). Posttranslational cleavage of the signal sequence after amino acid 25 would result in a 228-amino-acid polypeptide with an N-terminal sequence identical to that previously determined for oncostatin M (40). The encoded precursor sequence contained two potential N-glycosylation sites at residues 100 and 227 (Fig. 1) and five cysteine residues. Hydropathy analysis predicted that the oncostatin M precursor contains several strongly hydrophilic regions (18). Near the C terminus of the precursor were several paired dibasic amino acid residues that may represent potential proteolytic cleavage sites. A search of the amino acid sequence library of the Protein Identification Resource (release 18.0, September 1988) and the EMBL (release 17, November 1988) and GenBank (release 58, December 1988) nucleic acid sequence data bases showed no significant protein sequence homology to oncostatin M. The oncostatin M cDNA sequence contained an A+T-rich region at the 3′ untranslated end (~1,700 to 1,814 bp; Fig. 1). This region contained several ATTTA pentamer motifs and a TTATTTAT octamer motif found in the 3′ untranslated region of many other cytokine and lymphokine cDNAs (6, 27, 39).

The exon structure of the oncostatin M gene was determined by partial sequencing of the genomic clone, pOMH9 (Fig. 1 and 2). The intron-exon junction sequences conform to the GT. . .AT rule for nucleotides flanking eucaryotic exon boundaries. Three exons that contained the entire coding region of oncostatin M precursor were identified. The

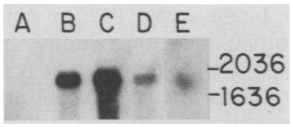

FIG. 3. Northern blot analysis of U937 and normal human T-cell poly(A) RNA. Poly(A) RNA, isolated from the indicated cell types, was probed with a 1.5-kbp ^{32}P-labeled fragment extending from the *Bgl*II site through the 3′ end of the oncostatin M cDNA. Lanes: A, untreated U937 cells; B to D, U937 cells treated with PMA for 16, 36, and 52 h, respectively; E, phytohemagglutinin-activated peripheral blood T lymphocytes. Positions of DNA size standards (in base pairs) are shown on the right.

first exon contained 5′ noncoding DNA and DNA encoding the initiating methionine and 10 amino acids of the hydrophobic signal sequence. The second exon of 143 nucleotides encoded the remainder of the hydrophobic region and the N-terminal 34 amino acids of the mature oncostatin M. The final exon encoded the remaining 193 amino acids of the mature polypeptide and contained all of the 3′ noncoding sequences present in the cDNA clone. The third exon is notable in that it contained almost 80% of the precursor-coding region and more than 1 kbp of 3′ noncoding sequences.

RNA blot analysis. Poly(A) RNA isolated from either U937 cells that had been treated with PMA for various times or activated T cells was subjected to Northern (RNA) blot analysis (Fig. 3). Under these conditions, we detected an mRNA species of ~2 kbp, which is consistent with the length of the cDNA sequence shown in Fig. 1. Oncostatin M mRNA was not detected in untreated U937 cells (Fig. 3, lane A) but accumulated in a time-dependent fashion between 16 and 36 h of PMA treatment (lanes B and C) and then decreased substantially after 52 h of treatment (lane D). Phytohemagglutinin-activated peripheral blood T lymphocytes, which are known to produce oncostatin M (5), also contained a ~2-kbp oncostatin M mRNA species (lane D). In some experiments, an additional species of ~1 kbp hybridized with oncostatin M cDNA probes (data not shown). The identity of this species remains to be determined.

Expression of oncostatin M in COS cells. The complete oncostatin M precursor-coding sequence was reconstructed in an expression vector, and the resulting construct, pSPOM, was transfected into COS cells as described in Materials and Methods. Serum-free culture medium was isolated from transfected cells and tested for the growth-inhibitory activity of oncostatin M (Fig. 4) and for the presence of oncostatin M protein by immunoblot analysis (Fig. 5).

Medium from cells transfected with pSPOM inhibited the proliferation of A375 melanoma cells, with a volume of approximately 1 μl being required for half-maximal inhibition of proliferation (Fig. 4). In contrast, medium from cells transfected with the vector alone (πH3MPy) did not significantly inhibit proliferation at volumes of up to approximately 10 μl. Medium from COS cells transfected with pSPOM contained two protein species of M_r 32,000 and 36,000 that reacted specifically with an antiserum prepared against a peptide corresponding to amino acid residues 31 to 44 of the oncostatin M precursor (Fig. 5A, lane 2). Analysis of cellular extracts from these transfected cells demonstrated the presence of smaller amounts of a lower-molecular-weight intracellular protein species (Fig. 5A, lane 1). All of these species were immunologically related to oncostatin M, as shown by the ability of the peptide encompassing amino acids 31 to 44

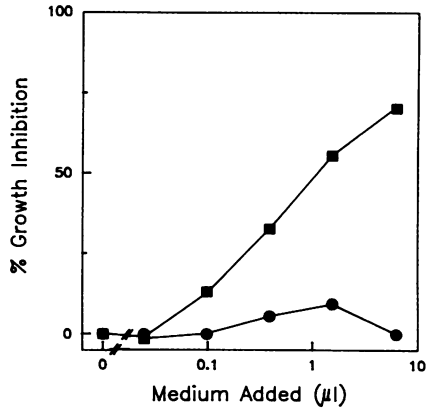

FIG. 4. Growth-inhibitory activity in medium from transfected COS cells. The indicated volumes of medium from COS cells transfected with the πH3MPy vector alone (●) or with pSPOM (■) were diluted to a final volume of 200 μl and tested for growth-inhibitory activity on A375 cells as described in Materials and Methods. Cellular proliferation was measured after 72 h and is expressed as percent growth inhibition, determined as $100\times$ [1 − (A_{590} treated)/(A_{590} untreated)].

to inhibit binding of the antiserum (Fig. 5B, lanes 1 and 2). All immunoreactive proteins migrated slightly slower than oncostatin M purified from U937 cells (M_r 28,000; Fig. 5C). No immunoreactive species were detected from COS cells transfected with πH3MPy alone (Fig. 5A, lanes 3 and 4).

The M_r-32,000 form of recombinant oncostatin M (Fig. 5A, lane 2) was purified by sequential size fractionation and

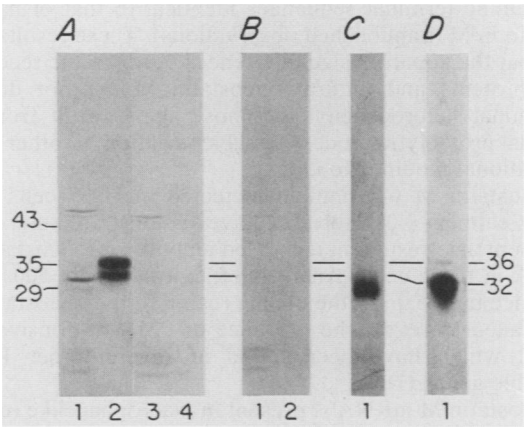

FIG. 5. Analysis of recombinant oncostatin M expressed in transfected COS cells. Medium and cell extracts from transfected COS cells or samples of purified oncostatin M were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Proteins were detected by immunoblot analysis (A to C) or by silver staining (D) as described in Materials and Methods. Electrophoretic mobilities were estimated by comparison with molecular weight standards (ovalbumin, 43,000; lactate dehydrogenase, 35,000), and carbonic anhydrase, 29,000) (shown on the left). Positions of the M_r 32,000 and 36,000 recombinant oncostatin M proteins are indicated on the right. (A) Cell extracts (lanes 1 and 3) or medium (lanes 2 and 4) from cells transfected with pSPOM (lanes 1 and 2) or πH3MPy vector alone (lanes 3 and 4). (B) Cell extracts (lane 1) or medium (lane 2) from cells transfected with pSPOM and analyzed as for panel A except that the antiserum was preincubated with 100 μg of its cognate peptide. (C) Oncostatin M purified from U937 cells (~500 growth-inhibitory units). (D) Purified M_r-32,000 oncostatin M species (~1 μg) from medium of COS cells transfected with pSPOM.

reversed-phase high-performance liquid chromatography (Fig. 5D). Approximately 50 pg of purified protein, as determined by amino acid analysis, was required to inhibit proliferation of A375 cells by 50%. A sample of this preparation was subjected to amino-terminal microsequence analysis, and a single sequence corresponding to the amino terminus of U937 oncostatin M (40) was obtained. Thus, not only was the recombinant M_r-32,000 species immunologically cross-reactive with natural oncostatin M, but it also had an identical amino-terminal sequence. The absence of the hydrophobic N-terminal sequence of the oncostatin M precursor suggests that it serves as a signal to provide for secretion of oncostatin M and is removed during this process.

DISCUSSION

We have described the molecular cloning and expression of the growth regulator oncostatin M. COS cells transfected with DNA encoding oncostatin M expressed growth-inhibitory protein(s) having the same biological activity initially ascribed to oncostatin M from activated U937 cells (40). Medium from transfected cells contained two proteins (M_r 32,000 and 36,000) that were immunologically related to oncostatin M purified from U937 cells but appeared slightly larger when analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. In an experiment similar to that described above, COS cells were also transfected with DNA encoding oncostatin M in the πH3MPy vector, in which the oncostatin M signal sequence was replaced by the signal sequence from simian transforming growth factor β1 (34). Similar protein species of M_r 32,000 and 36,000 were also detected in the media from these cells and were shown to have amino-terminal sequences identical to that of natural oncostatin M (unpublished observations). These results suggest that the apparent size differences between the recombinant proteins and natural oncostatin M are not due to N-terminal heterogeneity and most likely result from C-terminal proteolytic processing, glycosylation, or other post-translational modification(s).

Oncostatin M was initially detected in U937 cells after PMA treatment (40). Here, we have demonstrated that PMA treatment also results in increased amounts of oncostatin M mRNA in these cells. Analysis of approximately 500 bp of sequence upstream of the coding region of the oncostatin M gene failed to reveal the presence of PMA-responsive elements, which have been found in several other PMA-inducible genes (1).

Oncostatin M mRNA is present in macrophagelike (differentiated U937) cells and activated T lymphocytes. In recent experiments, we have also found oncostatin M mRNA in activated human monocytes (K. Grabstein and T. Rose, unpublished observations). Mononuclear cells are known to produce numerous other cytokines and lymphokines, many of which share the property of directly regulating cellular growth in vitro (7, 10, 24, 31, 36). Oncostatin M was originally identified as a growth inhibitor for a few human tumor cell lines (40); however, more recent studies with recombinant oncostatin M have shown that it may inhibit or stimulate cell proliferation or affect cell morphology of a wide variety of cultured normal and tumor cells (D. Horn, W. C. Fitzpatrick, P. T. Gompper, V. Ochs, M. Bolton-Hanson, J. Zarling, N. Malik, G. J. Todaro, and P. S. Linsley, submitted for publication). Thus, oncostatin M is one of many factors released by lymphocytes and macrophages that can function as a growth regulator.

In addition to being expressed in cells that produce cytokines and lymphokines, oncostatin M has the general structural features of a cytokine; i.e., it is a low-molecular-weight secreted glycoprotein. Oncostatin M also shares limited homology with a family of cytokines that affect hematopoietic stem cells (32). These cytokines have an N-terminal consensus sequence of Ala-Ser-Xaa-Ser or Ala-Pro-Xaa-Arg (or Ser)-Ser (or Leu). Oncostatin M has the terminal alanine and the serine at position 5 in common with this sequence. Other features of oncostatin M include its hydrophilic character and the presence of five cysteines, indicating the potential for at most two intrachain disulfide linkages. No significant homologies were noted between the amino acid sequence of oncostatin M and that of any other known protein, which suggests that it is a novel cytokine.

Although the amino acid sequence of oncostatin M has little homology to other known cytokines or lymphokines, its mRNA contains an A+U-rich region at the 3′ untranslated end that is homologous to a region found in the mRNAs of many cytokines, lymphokines, and other molecules, such as proto-oncogenes, that are involved in cellular growth control (6, 26, 27, 35). Current evidence suggests that these A+U-rich regions function in posttranscriptional regulation of gene expression by controlling mRNA levels (4, 26, 33, 35, 38). In some cases, these sequences appear to be involved in determining mRNA stability and may have evolved to regulate expression of proteins whose levels must be rapidly controlled (25). It is not yet known whether this homology is sufficient to destabilize oncostatin M transcripts, but the presence of these A+U-rich sequences in the 3′ untranslated region suggest that oncostatin M transcripts may share a common mode of regulation with transcripts of other cytokines and lymphokines.

In conclusion, oncostatin M is a structurally unique cytokine that specifically binds to and regulates the growth of a wide variety of cells. Although the in vivo function(s) of this molecule is presently unknown, the availability of recombinant oncostatin M derived from molecular clones described in this paper should facilitate future studies of its function, regulation, and possible therapeutic usefulness.

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