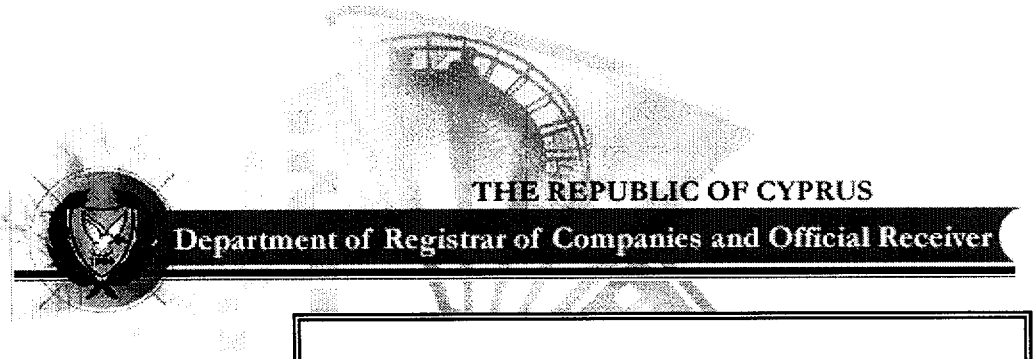


ΚΥΠΡΙΑΚΟ ΓΡΑΦΕΙΟ ΔΙΠΛΩΜΑΤΩΝ ΕΥΡΕΣΙΤΕΧΝΙΑΣ

THE PATENT OFFICE OF CYPRUS

ΑΡΙΘΜΟΣ ΔΗΜΟΣΙΕΥΣΗΣ **CY1608**
PUBLICATION NUMBER

ΑΡΙΘΜΟΣ ΔΗΜΟΣΙΕΥΣΗΣ
ΓΡΑΦΕΙΟΥ ΔΙΠΛΩΜΑΤΩΝ ΕΥΡΕΣΙΤΕΧΝΙΑΣ
ΗΝΩΜΕΝΟΥ ΒΑΣΙΛΕΙΟΥ
UK PATENT OFFICE
PUBLICATION NUMBER GB2185485

Το έγγραφο που παρουσιάζεται πιο κάτω καταχωρήθηκε στο «Γραφείο Διπλωμάτων Ευρεσιτεχνίας» στην Αγγλία σύμφωνα με το Νόμο Κεφ. 266 πριν την 1η Απριλίου 1998. Δημοσίευση έγινε μετέπειτα από το Γραφείο Διπλωμάτων Ευρεσιτεχνίας του Ηνωμένου Βασιλείου μόνο στην Αγγλική γλώσσα.

The document provided hereafter was filed at "The Patent Office" in England under the law CAP.266 before the 1st of April 1998. It was published afterwards by the UK patent office only in English.

(12) UK Patent Application (19) GB (11) 2 185 485 (13) A

(43) Application published 22 Jul 1987

(21) Application No 8629997

(22) Date of filing 16 Dec 1986

(30) Priority data
(31) 811235 (32) 20 Dec 1985 (33) US
935283 26 Nov 1986

(51) INT CL⁴
C07K 15/06

(52) Domestic classification (Edition I):
C3H F5
U1S 1313 2411 2416 C3H

(56) Documents cited
None

(58) Field of search
C3H
Selected US specifications from IPC sub-classes C07C C07G C07K

(71) Applicant
Oncogen

(Incorporated in USA—Washington)

3005 First Avenue, Seattle, Washington, United States of America

(74) Agent and/or Address for Service
Langner Parry,
52—54 High Holborn, London WC1V 6RR

(72) Inventors
Joyce M. Zarling
Mohammed Shoyab
Hans Marqardt
Marcia B. Hanson
Mario N. Lioubin
Thomas Joseph Brown

(54) Cell growth regulatory factor

(57) The factor is obtainable from leukocytes and is capable of inhibiting neoplastic cells, stimulating normal fibroblasts while not inhibiting T cell responses or colony formation by bone marrow cells. It has a molecular weight of 17—19 kD (gel exclusion chromatography) or 28 kD (SDS—PAGE), is relatively insensitive to acid or base and a temperature of 56°C.

The 35-mer N-terminal sequence and two internal 20-mer sequences are disclosed. Peptides of at least eight aminos acids which are immunologically cross-reative with the factor are claimed.

FIG. 1

1 5 10 15 20
A-A-I-G-S-C-S-K-E-Y-R-V-L-L-G-Q-L-Q-K-Q

25 30 35
T-D-L-M-Q-D-T-S-T-L-L-T-P-Y-I

1 5 10 15 20
Q-R-L-P-K-A-Q-D-L-E-R-S-G-L-N-I-E-D-L-E-K

1 5 10 15 20
L-R-E-H-C-R-E-R-P-G-A-F-P-S-E-Q-Q-L-I-G

GB 2 185 485 A

The drawing(s) originally filed was/were informal and the print here reproduced is taken from a later filed formal copy.

This print embodies corrections made under Section 117(1) of the Patents Act 1977.

2185485

FIG. 1

```
1         5         10        15        20
A-A-I-G-S-C-S-K-E-Y-R-V-L-L-G-Q-L-Q-K-Q

          25        30        35
T-D-L-M-Q-D-T-S-T-L-L-T-P-Y-I
```

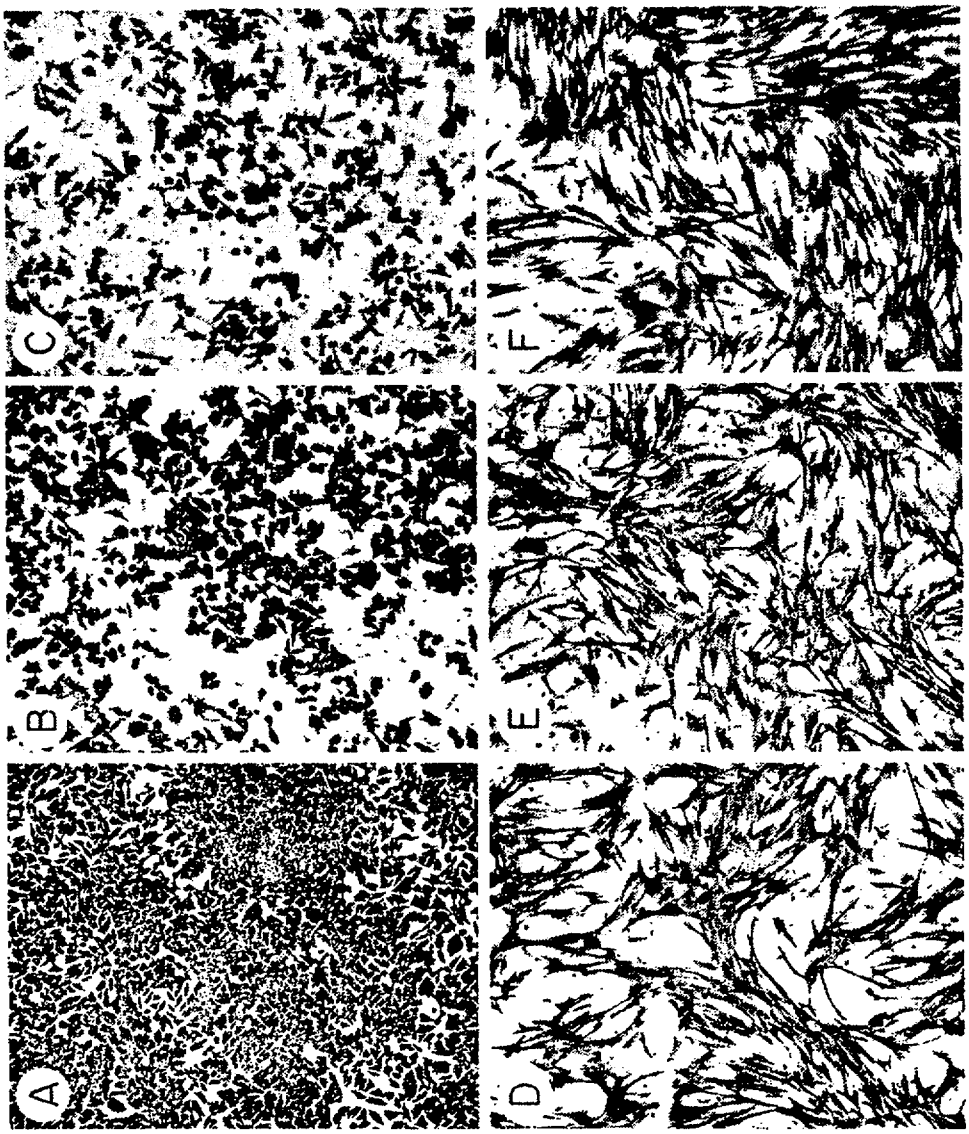
```
1         5         10        15        20
Q-R-L-P-K-A-Q-D-L-E-R-S-G-L-N-I-E-D-L-E-K
```

```
1         5         10        15        20
L-R-E-H-C-R-E-R-P-G-A-F-P-S-E-Q-Q-L-I-G
```

16 DEC '86 29997

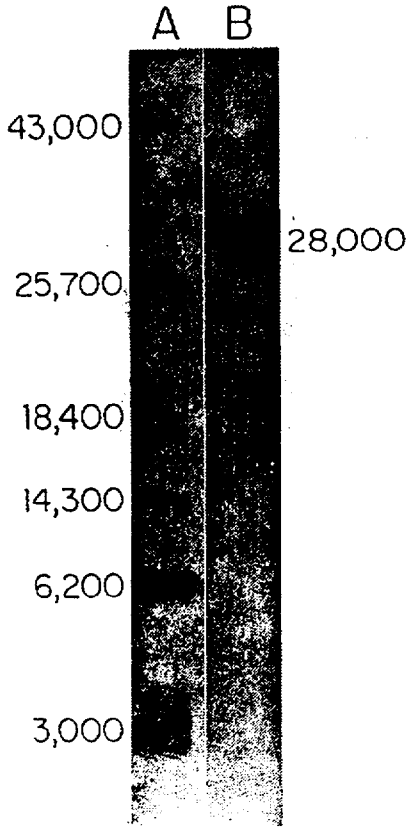
2185485

FIG. 2



2185485

FIG. 3



SPECIFICATION

Novel cell growth regulatory factor

BACKGROUND OF THE INVENTION

Field of the Invention

Leukocytes, both lymphocytes and monocytes, have been implicated in the inhibition of tumor growth in several animal tumor models. The increased incidence of malignancies in immunocompromised humans supports the contention that the white blood cells play a role in the regulation of neoplastic growth. Protein factors produced by these white cells that inhibit tumor growth or modulate immune functions which have already been isolated and characterized include the interferons, α- and γ-, tumor necrosis factor, lymphotoxin, interleukin-2, and other lymphokines. Since each of the factors which have been isolated have a different spectrum of activities and may interact differently in conjunction with other factors, there remains a continuing and strong interest in the isolation of and characterization of all of the factors which white cells produce in the modulation of cell growth or immune functions. These compounds individually or together may find use in the treatment or diagnosis of cancer, as promoters of wound healing or as immunomodulators for the treatment of patients with immunodeficiencies, autoimmunity, organ transplants, and the like.

There are several difficulties that may be encountered in the discovery, isolation, purification, or characterization of naturally occurring factors. Methodologies must be developed for separating and purifying a factor of interest from other factors in the crude starting material without denaturing the activity of the desired factor; bioassays must be developed which allow for identifying the fractions during separations which concentrate a particular factor; a novel factor must be distinguished from factors which are already known or other unknown factors which may be present and may affect, either negatively or positively, the activity of the factor being pursued; the purified factor must be characterized; and the purified factor must be concentrated in sufficient amount to permit identification and characterization of the factor. Therefore, with the increasing number of factors which have been isolated, each new factor becomes more difficult to identify, since its role and function may be obscured by the numerous other factors which are present.

Description of the Prior Art

Beal *et al., Cancer Biochem. Biophys.* (1979) *3*: 93–96 report the presence of peptides in human urine which inhibit growth and DNA synthesis more in transformed cells than in normal cells. Holley *et al., Proc. Natl. Acad. Sci.* (1980) *77*: 5989–5992 describe the purification of epithelial cell growth inhibitors. Letansky, *Biosci. Rep.* (1982) *2*: 39–45 report that peptides purified from bovine placenta inhibit tumor growth and thymidine incorporation in DNA to a greater extent in neoplasms than in normal cells. Chen, *Trends Biochem. Sci.* (1982) *7*: 364–365 reports the isolating of a peptide from ascites fluid with a cancer suppressing property. Redding and Schally, *Proc. Natl. Acad. Sci.* (1982) *79*: 7014–7018 report isolation of purified peptide(s) from porcine hypothalmi which exhibit antimitogenic activity against several normal and tumor cell lines. Sone *et al., Gann* (1984) *75*: 920–928 report the production of a factor(s) produced by human macrophages that inhibit the growth of certain tumor cells *in vitro*. Ransom *et al., Cancer Res.* (1985) *45*: 851–862, report the isolation of a factor called leukoregulin that inhibits replication of certain tumor cell lines and appears distinct from lymphytoxin, interferon and interleukin 1 and 2. Most of these factors have not been fully characterized, nor are their primary structures known.

Aggarwal *et al., J. Biol. Chem.* (1984) *259*: 686–691 purified and characterized human lymphotoxin (LT) produced by a lymphblastoid cell line and subsequently sequenced LT (Aggarwal *et al., J. Biol. Chem.* (1985) *260*: 2334). Gamma interferon (γ-IF) which is produced by lymphoid cells and has immunomodulatory and tumor inhibitory activity has been cloned and expressed. (Gray *et al., Nature* (1982) *295*: 503: 508.) Tumor necrosis factor (TNF), which inhibits growth of some tumors and is produced by macrophages and certain leukemia cell lines has been characterized and the TNF cDNA was cloned and expressed in *E. coli* (Pennica *et al., Nature* (1984) *312*: 724).

SUMMARY OF THE INVENTION

A novel peptide factor and biologically active fragments thereof are provided, which factor is available from leukocytes. The factor finds use in modulating cell growth, such as inhibiting tumor cell growth and stimulating growth of normal fibroblasts and may modulate immune functions. The factor has an amino acid sequence distinctively different from the sequences of other compounds which have been reported to have analogous properties.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 represents the amino acid sequence of fragments of Oncostatin M;
Figure 2 is a series of photomicrographs of cells treated with Oncostatin M wherein (A-C) are A375 melanoma cells treated with 0, 5 and 100 GIA units, respectively, and (D-F) are WI38 (fibroblasts treated with 0, 5 and 100 GIA units, respectively; and
Figure 3 is a photograph of an SDS-PAGE analysis of Oncostatin M.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

A novel polypeptide, polypeptide compositions, polypeptide fragments and mutations, their preparation and use are provided, with the compositions demonstrating activity in the modulation of cell growth, particularly inhibiting tumor cell growth and stimulating growth of normal fibroblasts. A subject polypeptide referred to as Oncostatin M is available from leukocytes; e.g., from conditioned media of stimulated U937 cells or conditioned media of stimulated normal human peripheral blood lymphocytes (PBL). Fragments and mutants of the polypeptide having the biological activity of the intact Oncostatin M such as cell growth modulation activity of immunologic activity are also provided.

The polypeptide fragments of this invention are novel polypeptides of at least 8 amino acids that are biologically active, at least as to being immunologically cross-reactive with naturally occurring Oncostatin M. By immunologically cross-reactive it is meant that an antibody induced by a novel polypeptide of this invention will cross-react with intact Oncostatin M at least when Oncostatin M is in a denatured state. Those polypeptides are therefore useful to induce antibodies to Oncostatin M which can be used to determine the concentration of Oncostatin M in a bodily fluid, to bind the Oncostatin M and thus modulate its activity, and to purify Oncostatin M, as by use in an affinity column. A portion of the polypeptides may also retain the cell growth modulatory activity of intact Oncostatin M, although that activity may be modulated, usually reduced in comparison to intact Oncostatin M.

Fig. 1 represents the amino acid sequence of poly(amino acid)s cross-reaction with Oncostatin M with the first sequence representing the N-terminus of Oncostatin M.

Poly(amino acid)s of the present invention contain an amino acid sequence having at least 8 consecutive amino acids that correspond to an amino acid sequence depicted in Fig. 1 and differing from that sequence by no more than 3, usually no more than 1 amino acid. That difference can be either the insertion of an amino acid, the deletion of an amino acid or the substitution of one amino acid for another, particularly a conservative substitution. Usually the poly(amino acid)s will contain at least 10, more usually at least 12, consecutive amino acids that correspond to sequences depicted in the figure and differ by no more than one amino acid.

For purposes of the subject invention, the various amino acids will be divided into a number of subclasses. The following table indicates the subclasses:

aliphatic							
neutral							
non-polar	G	A	P	V	L	I	
polar	S	T	C	M	N	Q	
acidic	D	E					
basic	K	R					
aromatic	F	H	Y	W			

By conservative substitution, it is meant that amino acids from the same subclass (i.e., either neutral aliphatic, acidic aliphatic, basic aliphatic or aromatic), more particularly the same polarity, will be substituted for each other. Desirably, amino acids of two to four carbon atoms or five to six carbon atoms will define monomer groupings in the aliphatic subclass.

The poly(amino acid)s will not exceed about 1000 amino acids in length. Usually they will have fewer than one hundred amino acids, more usually fewer than fifty amino acids. Thus, the poly(amino acid)s can be readily synthesized. Usually when poly(amino acid)s exceed 100 amino acids in length, those poly(amino acid)s may be polymers of fragments of Oncostatin M having fewer than 100 amino acids each, or fusion proteins where the fragment is fused to an antigen, enzyme, enzyme fragments, etc. Particularly, the higher molecular weight poly(amino acid)s can be at least one polypeptide fragment of fewer than about 100 amino acids joined covalently to a large immunogenic polypeptide carrier to provide for immunogenicity. Exemplary of such protein carriers are bovine serum albumin, keyhole limpet hemocyanin (KLH) and the like. Those conjugated polypeptides will be useful for inducing antibodies in an appropriate host organism.

U937 cells are a cell line derived from a histiocytic lymphoma cell line (Sundstrom and Nilsson, *Int. J. Cancer* (19760 *17*: 565–577) that can be induced to differentiate into cells having characteristics of macrophages following treatment with a variety of agents (Harris *et al.*, *Cancer Res.* (1985) *45*: 9–13). For production of Oncostatin M, the U937 cells may be grown in a conventional nutrient medium with serum and treated with an appropriate inducer. Conveniently, phorbols or ingenols may be employed, particularly 12-O-tetradecanoylphorbol-13-acetate (TPA).

Usually, from about 5–20ng/ml of the inducer may be employed. The initial number of cells is from about 10^5–10^6 cells/ml.

After allowing cells to be treated with the inducer for a sufficient time, generally three to six days, the supernatant is removed, the cells washed with serum-free nutrient medium, attached cells washed again with serum-free medium and the cells allowed to incubate for at least 12 hours, usually not more than about 48 hours, in serum-free nutrient medium, e.g. RPMI-1640 medium. Supernatants are then collected and cells are removed by centrifugation. Cell-free supernatants were tested for cell growth inhibitory activity (GIA) as described in the experimental plan. The supernatant contains about 50 to 500 units of GIA/ml (see Experimental for definition of GIA units.)

Oncostatin M can also be obtained from mitogen-stimulated normal human peripheral blood lymphocytes (PBL's). PBL's can be isolated from leuko-fractions by diluting the fractions and centrifuging them over Ficoll gradients. Cells collected from the gradient interface are washed and shock-lysed to remove red blood cells. Remaining cells are collected from that solution by centrifugation, resuspended in buffer containing serum and thrombin, agitated and the platelet aggregate allowed to settle for a short period of time. The suspended cells are transferred, recovered by centrifugation, resuspended in serum and transferred to a column containing nylon wool. The column is incubated to allow the attachments of monocytes and B-lymphocytes and then washed. Most peripheral blood T lymphocytes do not adhere and are eluted from the column. Those cells were cultured at 37° in culture medium, e.g. RPMI-1640 medium, and treated with an appropriate inducer, e.g. phytohemagglutinin (about 1 to 5 mg/l), for about 100 hours and then supernatants were collected. The supernatants were centrifuged to remove cells and concentrated as by ultrafiltration or dialysis.

After isolating cell-free supernatant from either U937 cells or normal PBL's, the conditioned medium is concentrated, conveniently using a hollow fiber system or an ultrafiltration membrane, followed by dilution with acetic acid (to a concentration of 0.1N acetic acid) followed by concentrating about ten-fold and the dilution and concentration repeated. The concentrate may be lyophilized and used directly or the lyophilized product can be used for further purification.

The subject Oncostatin M can be purified by a procedure of gel permeation chromatography using aqueous 40% acetonitrile-0.1% trifluoroacetic acid as an isocratic mobile phase on a Bio-sil TSK250 column, monitoring activity for each of the fractions. Purification provides for a composition having at least about $0.5–5 \times 10^4$ GIA units/ml in the active fractions.

The partially purified product from the gel permeation chromatography may be further purified by employing reverse phase high-pressure liquid chromatography employing a linear gradient, where the primary solvent is 0.1% trifluoroacetic acid in water and the secondary solvent is acetonitrile containing 0.1% trifluoroacetic acid. The schedule can be varied, generally the chromatographing requiring about 3–4 hours, with the major portion of the time, greater than about 50% of the time and not more than about 80% of the time, in the range of 30–45% of the secondary solvent. Under these conditions the active fractions elute at about 41–42% acetonitrile.

The pooled active fractions may be further purified by repeating the reverse phase HPLC, employing a more rapid change in the gradient conditions and a slower flow rate. Under these conditions, the activity emerges at about 40.5–41.5% acetonitrile.

The reverse phase HPLC may then be repeated, changing the solvent system, where the secondary solvent is *n*-propanol-0.1% trifluoroacetic acid. A linear gradient is employed, where the gradient is changed slowly in the range of 23–35% *n*propanol. The major activity is observed in the range of 25.5–27.5% propanol, to give a substantially homogeneous product, having a specific activity of greater than 10 GIA units/ng protein. Usually, the product is purified to provide a specific activity of at least about 100 GIA units/ng protein, more usually 150 GIA units/ng.

The subject compounds are characterized by having a molecular weight of about 17 to 19 kiloDaltons (kD), particularly about 18kD, as determined by size exclusion chromatography The subject compounds are further characterized as having an apparent molecular weight of approximately 28kD as determined by polyacrylamide gel electrophoresis under reducing or non-reducing conditions.

The amino acid sequence of fragments of purified Oncostatin M was analyzed. Oncostatin has substantially the amino acid sequences represented in Fig. 1. Referring to Fig. 1, the first sequence illustrates the N-terminus of Oncostatin M, while the remaining sequences illustrate internal fragments of the polypeptide.

Active preparations of isolated Oncostatin M contained a mixture of high mannose and complex N-linked oligosaccharide. However, non-glycosylated preparations of Oncostatin M retained cell growth modulatory activity.

Oncostatin M is further characterized by its activity toward certain cell strains. The subject polypeptide lacks cytotoxic activity against WI26 and WI38 human fibroblasts, and mouse L929 cells which are sensitive to tumor necrosis factor, and a γ-interferon-sensitive human tumor cell

line. It also is found not to inhibit proliferation of normal human T-lymphocytes or to inhibit granulocytic/myelocytic colony formation from bone marrow cells at concentrations up to 100 GIA units/ml. Further, Oncostatin M stimulates proliferation of normal human fibroblasts as exemplified by WI38 and WI26 cells and inhibits proliferation of tumor cells such as A375, HBT10, A549 and SK-MEL28 and may augment growth of colony forming cells from normal bone marrow. Oncostatin M did not suppress human proliferative or cytotoxic T cell responses in mixed leukocyte culture reactions (MLC) at concentrations of 500 GIA units/ml.

The subject polypeptide is found to be stable to moderate acid and base and to heat treatment at 56°C.

The amino acid sequence of the subject polypeptide may be completely determined using commercially available sequencers. The polypeptide may then be synthesized in accordance with known techniques, employing automated synthesizers which are also commercially available.

Alternatively, the subject polypeptide may be produced by recombinant DNA techniques. From a partial amino acid sequence, probes can be deduced which can then be used for screening a human genomic library. The library may be a cDNA library or a chromosomal library. Once the clone(s) have been identified as annealing to the probe, the fragments containing the gene of interest may be identified in a number of ways and manipulated in a number of ways. The fragment may be reduced in size by endonuclease restriction, with the resulting fragments cloned and probed for the presence of the desired gene. Cells which produce the desired peptide or produce enhanced amounts of the desired peptide may be employed for production of messenger RNA. From the messenger RNA, single stranded cDNA may be prepared. The cDNA may then be annealed to total messenger RNA from a cell which produces little, if any, of the polypeptide. The unannealed cDNA may then be isolated and used to prepare ds cDNA, which may be screened with the probes.

Alternatively, DNA fragments may be inserted into λgt11, so that coding fragments may be downstream from and in frame with the β-galactosidase gene. Antibodies may be prepared to the Oncostatin M polypeptide and used for screening the resulting fused proteins for cross-reactivity. In this manner, fragments coding for the subject polypeptide or fragment thereof may be identified and used for identifying the desired gene.

Once a complete gene has been identified, either as cDNA or chromosomal DNA, it may then be manipulated in a variety of ways to provide for expression. Where the gene is to be expressed in a host which recognized the wild-type transcriptional and translational regulatory regions of Oncostatin M, the entire gene with its wild-type 5'- and 3'-regulatory regions may be introduced into an appropriate expression vector. Various expression vectors exist employing replication systems from mammalian viruses, such as Simian Virus 40, adenovirus, bovine papilloma virus, vaccinia virus, insect baculovirus, etc. These replication systems have been developed to provide for markers which allow for selection of transfectants, as well as providing for convenient restriction sites into which the gene may be inserted.

Where the gene is to be expressed in a host which does not recognize the naturally occurring wild-type transcriptional and translational regulatory regions, further manipulation will be required. Conveniently, a variety of 3'-transcriptional regulatory regions are known and may be inserted downstream from the stop codons. The non-coding 5'-region upstream from the structural gene may be removed by endonuclease restriction, *Bal* 31 resection, or the like. Alternatively, where a convenient restriction site is present near the 5'-terminus of the structural gene, the structural gene may be restricted and an adaptor employed for linking the structural gene to the promoter region, where the adaptor provides for the lost nucleotides of the structural gene. Various strategies may be employed for providing for an expression cassette, which in the 5'-, 3'-direction of transcription has a transcriptional and translational initiation region, which may also include regulatory sequences allowing for the induction of regulation, the structural gene under the transcriptional and translational control of the initiation region, and a transcriptional and translational termination region.

Illustrative transcriptional initiation regions or promoters include, for bacteria, the β-gal promoter, the TAC promoter, lambda left and right promoters, etc.; for yeast, glycolytic enzyme promoters, such as ADH-I and -II promoters, GPK promoter, and PGI promoter, TRP promoter, etc.; for mammalian cells, SV40 early and late promoters, adenovirus major late promoters, etc. As already indicated, the expression cassette may be included within a replication system for episomal maintenance in an appropriate cellular host or may be provided without a replication system, where it may become integrated into the host genome. The DNA may be introduced into the host in accordance with known techniques, such as transformation, using calcium phosphate-precipitated DNA, transfection by contacting the cells with the virus, microinjection of the DNA into cells or the like.

Once the structural gene has been introduced into the appropriate host, the host may be grown and will express the structural gene. In some instances, it may be desirable to provide for a signal sequence (secretory leader) upstream from and in reading frame with the structural gene, which provides for secretion of the structural gene and cleavage of the secretory leader,

so as to provide for the mature polypeptide in the supernatant. Where secretion is not provided for, then the host cells may be harvested, lysed in accordance with conventional conditions, and the desired product isolated and purified in accordance with known techniques, such as chromatography, electrophoresis, solvent extraction, or the like.

The subject compounds can be used in a wide variety of ways, both *in vivo* and *in vitro*. The subject compounds can be used for making antibodies to the subject compounds, which may find use *in vivo* or *in vitro*. The antibodies can be prepared in conventional ways, either by using the subject polypeptide as an immunogen and injecting the polypeptide into a mammalian host, e.g. mouse, cow, goat, sheep, rabbit, etc., particularly with an adjuvant, e.g. complete Freunds adjuvant, aluminum hydroxide gel, or the like. The host may then be bled and the blood employed for isolation of polyclonal antibodies, or in the case of the mouse, the peripheral blood lmphocytes or splenic lymphocytes (B-cells) employed for fusion with an appropriate myeloma cell to immortalize the chromosomes for monoclonal expression of antibodies specific for the subject compounds.

Either polyclonal or monoclonal antibodies may be prepared, which may then be used for diagnosis or detection for the presence of the subject polypeptide in a sample, such as cells or a physiological fluid, e.g. blood. The antibodies may also be used in affinity chromatography for purifying the subject polypeptide and isolating it from natural or synthetic sources. The antibodies may also find use in controlling the amount of the subject polypeptide associated with cells in culture or *in vivo*, whereby growth of the cells may be modified.

The subject compound may be used as a ligand for detecting the presence of receptors for the subject compound. In this way, cells may be distinguished in accordance with the presence of and the density of receptors for the subject compound, monitoring the effect of various compounds on the presence of such receptors.

The subject compound may be used in *in vitro* cultures to inhibit the growth of cells or cell lines sensitive to the subject polypeptide as distinguished from cells which are not sensitive. Thus, heterogeneous cell mixtures or cell lines can be freed of undesirable cells, where the undesirable cells are sensitive to the subject polypeptide. The subject polypeptide may be administered *in vivo* in the case of neoplastic conditions, for example, by injection, intralesionally, peritoneally, subcutaneously, or the like. The subject compound may be used *in vitro* to eliminate malignant cells from marrow for autologous marrow transplants or to inhibit proliferation or eliminate malignant cells in other tissue, e.g. blood, prior to reinfusion.

The subject polypeptide may also be used in a method for treating wounds, such as cutaneous wounds, corneal wounds, and various other epithelial and stromal disruptions, such as chronic ulcers, burns, surgical incisions, traumatic wounds, and injuries to the hollow, epithelial-lined organs, such as the esophagus, stomach, large and small bowels, mouth, genital, and urinary tract. The method relies on the topical application of a treatment composition including Oncostatin M in a physiologically-accepable carrier.

The composition of the present invention may be used for treating a wide variety of wounds including substantially all cutaneous wounds, corneal wounds, and injuries to the epithelial-lined hollow organs of the body. Wounds suitable for treatment include those resulting from trauma such as burns, abrasions, cuts, and the like as well as from surgical procedues such as surgical incisions and skin grafting. Other conditions suitable for treatment with the compositions of the present invention include chronic conditions, such as chronic ulcers, diabetic ulcers, and other non-healing (trophic) conditions.

Oncostatin M may be incorporated in physiologically-acceptable carriers for application to the affected area. The nature of the carriers may vary widely and will depend on the intended location of application. For application to the skin, a cream or ointment base is usually preferred, suitable bases include lanolin, Silvadene (Marion) (particularly for the treatment of burns), Aquaphor (Duke Laboratories, South Norwalk, Connecticut), and the like. If desired, it will be possible to incorporate Oncostatin M containing compositions in bandages and other wound dressings to provide for continuous exposure of the wound to the peptide. Aerosol applications may also find use.

The concentration of polypeptide in the treatment composition is not critical. The polypeptide will be present in an epithelial cell proliferation-inducing amount. The compositions will be applied topically to the affected area, typically as eye drops to the eye or as creams, ointments or lotions to the skin. In the case of eyes, frequent treatment is desirable, usually being applied at intervals of 4 hours or less. On the skin, it is desirable to continually maintain the treatment composition on the affected area during the healing, with applications of the treatment composition from two to four times a day or more frequently.

The amount employed of the subject polypeptide will vary with the manner of administration, the employment of other active compounds, and the like, generally being in the range of about 1μg to 100μg. The subject polypeptide may be employed with a physiologically acceptable carrier, such as saline, phosphate-buffered saline, or the like. The amount of compound employed will be determined empirically, based on the response of cells *in vitro* and response of

experimental animals to the subject polypeptides or formulations containing the subject polypeptides. The subject compounds find use by themselves or in combination with other growth factors or inhibitors or immunomodulators, such as TNF, IL-2, γ-interferon, monoclonal antibodies, etc. The amounts of these other compounds will generally be in the range of 1 μg to 100μg. Conjugates of the subject compounds to site directing moieties, e.g., antibodies may be prepared, where the antibodies may be specific for particular malignant cells or organs.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

MATERIALS AND METHODS

Oncostatin M Isolated from U937 Cells Production of a Tumor Cell Growth Inhibitor From Histiocyctic Lymphoma Cell Line

U937 cells, a histiocyctic lymphoma cell line (Sundstrom and Nilsson, *Int. J. Cancer* (1976) *17*: 565–577), were cultured in 850cm² roller bottles (Corning C2540) at a concentration of 4×10^5 cells/ml in a total volume of 300ml RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), penicillin/streptomycin (PS), L-glutamine and 10ng/ml 12-0-tetradecanoylphorbol 13-acetate (TPA). Four days later, the supernatants containing the FCS and TPA were removed, the roller bottles were washed five times with serum-free RPMI-1640, and the cells which detached (1×10^5 cells/ml) were washed 3 times with serum-free medium and added back to the bottles, resulting in a final volume of 125ml serum-free RPMI-1640 medium per roller bottle. One day later, the supernatants were collected, centrifuged to remove the cells, filtered through 0.45 micron (μ) Nalgene filter and concentrated using a hollow fiber system (Amicon cartridge HIP10–20) to a volume of 150ml (initial volume 1500ml). Oncostatin M was also isolated from supernatants of serum-free TPA-treated U937 cells in 150 CM² tissue culture flasks. The supernatant was concentrated with an Amicon Diaflo membrane PM-10, 10kD cut-off and dialyzed. Following dialysis, the concentrate was diluted with acetic acid resulting in a final concentration of 0.1N acetic acid in 500ml and concentrated to 50ml using an Amicon PM 10 filter. The 50ml concentrate was diluted to 400ml with 0.1N acetic acid and concentrated to 40ml with the same filter. The concentrate was diluted with 1N acetic acid and the resulting precipitate was removed by centrifugation. The resulting concentrate was frozen and lyophilized. The lyophilized material was used for purification steps.

Gel Permeation Chromatography

A Bio-sil TSK-250 column (600×21.5mm) (Bio-Rad) was attached to a high pressure liquid chromatographic system. The crude fraction (10mg/ml) was dissolved in 40% acetonitrile in 0.1% aqueous trifluoroacetic acid (0.1% TFA). A 2ml aliquot of the mixture was injected and elution was performed isocratically with a mobile phase of 40% acetonitrile in 0.1% TFA. The flow rate was 2.5ml/min and chart speed was set at 0.25cm/min. 5ml fractions were collected. The chromatography was performed at room temperature. An aliquot from each fraction was evaporated and assayed in triplicate for growth inhibitory activity (GIA) of A375 cells.

The active fractions (Fractions 21 and 22) from six runs were pooled. The pooled material had a total of approximately 4.8×10^5 GIA units. The factor was found to have an apparent molecular weight of 18kD as determined by size exclusion chromatography (Bio-Sil TSK-250 column).

Reverse Phase High Pressure Chromatography (HPLC) of TSK-250 Fractions

Pooled TSK-250 fractions 21 and 22 described above were diluted two-fold with 0.1% TFA. This mixture was injected isocratically on a μ-Bondapak-C18 column (7.8×300mm) (referred to as C18[1]) at room temperature. The flow rate was set at 2.0 ml/min and the chart speed was 0.25 cm/min. The linear gradient was used between primary solvent 0.1% TFA and the secondary solvent acetonitrile-TFA 0.1%. The gradient conditions were 0–30% in 20min; then 30–45% in 150min; 45–55% in 20min; and 55–100% in 10min. All solvents were HPLC grade. 4ml fractions were collected and aliquots of each fraction were assayed for growth inhibitory activity. Fractions 72–75 were found to contain the majority of activity. The active fractions eluted between 41–52% of acetonitrile concentration.

Fractions 72–75 were pooled. 16ml of 0.1% TFA was added to the pooled fractions. The mixture was injected into a μ-Bondapak-C18 column (3.9×300mm) (referred to as C18[2]) at room temperature. The flow rate was set a 1ml/min and chart speed was 0.25cm/min. The gradient conditions were 0–35% in 10min; 35–45% in 100min; and 45–100% in 10min. Fractions were collected and aliquots were taken and assayed for GIA. Most of the activity emerged from the column between 40.7 to 41.3% acetonitrile concentration (retention time 83–86min).

Active fractions were pooled and diluted two-fold with 0.1% TFA and injected isocratically on a μ-Bondapak-C18 column (3.9×300mm) (referred to as C18[3]) at room temperature. The flow rate was 1ml/min and chart speed was 0.25 cm/min. A linear gradient was used between primary solvent 0.1% TFA and the secondary solvent *n*-propanol-TFA (0.1%). The gradient conditions were 0–23% in 20min and 23–35% in 120min. Fractions were collected and aliquots

of each fraction were assayed for GIA. Most of the activity appeared between 25–26.5% propanol concentration (retention time 59min). This apparently homogeneous fraction contained approximately 300ng protein and about 40,000 GIA units.

Cell Growth Modulatory Assay Using ^{3}H-Thymidine Incorporation into DNA (GIA)

The assays were performed in 96 flat well plates (Costar 3596). Human melanoma cells (A375) were used as a sensitive indicator cell line. Cells (3×10^3) in 0.1ml Dulbecco's modified Eagles medium (DMEM) supplemented with 10% FCS and PS were placed in each well. Three hours later, 0.1ml of test samples was added to each well. Plates were incubated at 37°C for 3 days. Then 0.025ml (0.5μCi) of a solution of ^{3}H-thymidine (specific activity 27μCi/μg) was added to each well for the final 6 hours of incubation. The cells were then transferred to glass filter strips by using a multiwell harvester (PHD Cell Harvester, Cambridge Technology, Inc.). The filters were transferred to scintillation vials to which were added 2ml of scintillation fluid (Scientiverse II, Fisher Scientific Co.) prior to counting in a scintillation counter for quantitating ^{3}H-thymidine incorporation.

Soft Agar Colony Inhibition Assay (TGI)

A 0.5ml base layer of 0.5% agar (Agar Noble; Difco Laboratories, Detroit, Michigan) in DMEM containing 10% fetal calf serum (FCS) was added to 24 well Costar tissue culture plates. 0.5ml 0.3% agar containing the same medium-FCS mixture, $1–2.5\times10^3$ A375 cells and the factor to be tested at various concentrations were overlaid on the base layer of agar. The plates were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air and refed after 7 days by addition of 0.5ml of 0.3% agar containing the same medium and concentrations of factor. Colonies were enumerated unfixed and unstained and the number of colonies greater than 6 cells were scored between days 7 and 14.

Results

Sequences of Oncostatin M Isolated from U937 Cells

The N-terminal sequence and internal fragments of Oncostatin M were determined by microsequence analysis of the reduced and S-pyridinethylated polypeptide and of peptides obtained from enzymatic digests of reduced and S-pyridinethylated Oncostatin M with the endoproteinase Lys-C and *Staphylococcus aureus* V8 protease. The peptide fragments were purified by reverse phase HPLC, using volatile solvents. The peptides were subjected to automated repetitive Edman degradation in the Model 470A protein sequencer (Applied Biosystems, Inc.). The phenylthiohydantoin amino acids were analyzed by reverse phase HPLC (Applied Biosystems, Inc.) with a PTH-C18 column (2.1×220mm, ABI), using a sodium acetate buffer/tetrahydrofuran/acetonitrile gradient for elution.

The resultant amino acid sequences are substantially as illustrated in Fig. 1.

A comparison of these sequences with those stored in the curent protein data base (PIR Release 9.0, May 1986), revealed no significant sequence homologies with any other known sequence. In addition, there is no homology with tumor necrosis factor, lymphotoxin, colony stimulating factor, interleukin 1 or 2 or β-transforming growth factor.

Inhibition of Proliferation of Tumor Cells and Augmentation of Proliferation of Normal Human Fibroblasts

Employing the soft agar colony inhibition assay described above, the following results were obtained:

Table 1. Inhibition of A375 Melanoma Cell Colony Formation in Soft Agar by Purified Oncostatin M Isolated from U937 Cells*

GIA Units/Well		% Inhibition of # Colonies	Colony Formation
250	4	96	
83	6	94	
27	11	89	
45	32	69	
0	106	—	

*A375 cells were plated in soft agar, with or without factor in a final volume of 2ml, as described above. The factor used was from a C18 propanol column fraction with peak tumor growth inhibitory activity (GIA). Eleven days later the numbers of colonies were enumerated. A colony was defined as a cluster of at least 6 cells. One GIA unit was defined as the amount to cause 50% inhibition of ^{3}H-thymidine incorporation into A375 cells in micro wells as detailed above.

In the next study, various treatments were employed to determine the effect of the treatment, either chemical or physical, on the activity of the subject polypeptide. The following table indicates the results.

Table 2. Effect of Various Treatments of Supernatants of TPA-Induced U937 Cells on Tumor Growth Inhibitory Activity*

	Final Dilution of Supernatant			
	1:4	1:8	1:16	
Media Control	—	—	—	39,780
Untreated Super.	7,206**	13,896	16,000	
1N Acetic Acid	6,670	17,073	18,783	
1N_4OH	6,956	15,016	13,923	

*U937 cells were treated with TPA (10ng/ml) for 3 days and then the cells were washed with media and incubated for 24hr in serum-free media before collecting the supernatants. The supernatants were treated with 1N acetic acid or 1N ammonium hydroxide (NH_4OH). They were then dialyzed against medium and tested for their ability to inhibit ^{3}H-thymidine incorporation into A375 cells. A375 cells were labeled with ^{3}H-thymidine (^{3}H-TdR) for the last 6hr of a 3-day incubation.

**Data shown are ^{3}H-TdR incorporation counts per minute.

The above results demonstrate that Oncostatin M in the dialyzed supernatant is substantially resistant to inactivation by one normal acetic acid and one normal ammonium hydroxide. Thus, the subject compounds are relatively insensitive to both moderately strong acid and moderately strong base. The subject compound is stable to heat treatment at 56°C. for 1 hour but not 90°C. for 30 minutes.

The subject compound was also tested for heat stability and was found to retain its activity after exposure at 56°C for 1hr, but to lose substantially all its activity after exposure to 95°C for 30min.

In the next study, the ability of the subject polypeptides to inhibit tumor cell replication of a variety of neoplastic cells was investigated. The following table indicates the results.

Table 3. Inhibition of Replication of Tumor Cells by Purified Oncostatin M from U937 Cells

Tumor Cells	Units GIA to Cause 30% Inhibition ^{3}H-TdR Incorporation
A549 (lung cancer)	21
HTB10 (neuroblastoma)	81
A375 (melanoma)	0.3

Tumor cells were plated in microwells 3hr prior to the addition of various dilutions of Oncostatin M, purified by reverse phase HPLC as detailed above. For the final 6hr of a 3-day incubation, cells in 0.2ml medium were labeled with ^{3}H-thymidine (^{3}H-TdR) (0.5μCi/well). One unit tumor growth inhibitory activity (GIA) is defined in the legend to Table 1 as that amount which causes 50% inhibition of ^{3}H-TdR incorporation into A375 melanoma cells. One unit was determined to be approximately 10pg of purified protein, therefore the concentration (ng/ml) to cause 30% inhibition ^{3}H-TdR into A549, HTB10 and A375 cells was approximately 1.4, 4.0, and .015ng/ml, respectively. ^{3}H-TdR incorporation into WI26 normal human fibroblasts was not suppressed by U937 factor in any experiment.

The above results demonstrate that Oncostatin M is selective in its ability to inhibit replication, having widely varying effects depending upon the nature of the cell. The subject compound is effective against melanoma cells, such as A375 melanoma cells, squamous lung cancer cells, such as A549, and neuroblastoma cells, such as HTB10.

Tumor cells were seeded at 3×10^3 cells/well and normal fibroblasts at 1.5×10^3 cells/well in 96 well plates for 3 hours. Various concentrations of purified Oncostatin M, obtained from the fraction from the C18^3 column with peak antiproliferative activity against A375 cells were added and three days later ^{3}H-thymidine incorporation into cells was measured in triplicate wells at each concentration. The results are shown in Table 4.

Table 4. Inhibition of Proliferation of Tumor Cells and Augmentation of Proliferation of Normal Fibroblasts by Oncostatin M

	GIA units/well	% inhibition		% stimulation
		A375		*WI38*
Exp. 1	16	83		25
	4	62		30
	1	46		46
		A375	*HTB10*	*WI26*
Exp. 2	27	NT	28	46
	9	87	22	36
	3	76	11	52
		A375	*A549*	
Exp. 3	75	89	30	
	25	85	22	
	8	71	16	
		A375	*Sk-MEL28*	
Exp. 4	20	87	44	
	5	75	25	
	1	59	11	

Results shown are % inhibition or % stimulation of ^{3}H-thymidine incorporated into tumor cells (A375, HTB10, A549, and SK-MEL28) and normal fibroblasts (WI26 and WI38), respectively. One GIA unit is defined in the legend to Table 1 as the amount of Oncostatin M that causes 50% inhibition of ^{3}H-thymidine incorporation into A375 cells.

In addition to the observed differential effect on ^{3}H-thymidine incorporation into tumor cells and normal human fibroblasts, there is also an observed differential effect of morphology and cell number following 3 days of treatment of the two cell types with Oncostatin M as shown in Fig. 2.

The Oncostatin M used was from the HPLC-C18^3 column fraction with peak activity for inhibiting proliferation of A375 cells. Fig. 2 is a series of photomicrographs of A375 melanoma cells that were untreated (A), treated with 5 growth inhibitory activity (GIA) units of Oncostatin M (B), or 100 units (C). Photomicrographs of WI38 fibroblasts that were untreated (D), treated with 5 units GIA (E), or 100 units (F). The cells were stained with crystal violet in 0.5% methanol. Magnification=63X.

$NaDodSO_4$/PAGE of Oncostatin M

Purified Oncostatin M, subjected to $NaDodSO_4$ performed under reducing conditions, was found to have an apparent molecular weight of approximately 28,kD as shown in Fig. 3. The following proteins were used as standards (lane A): ovalbumin, Mr=43 kD chymotrypsinogen α, Mr=25.7 kD; lactoglobulin β, Mr=18.4 kD; lysozyme, Mr−14.2 kD; bovine trypsin inhibitor=6.2 kD; insulin A and B chain, Mr=2.3 kD and 3.4 kD respectively. Oncostatin M was applied to lane B.

Oncostatin M, subjected to PAGE under non-reducing conditions, also has an apparent molecu-

lar weight of 28 kD and protein electroeluted from the band was found to inhibit proliferation of A375 cells.

Antibody to a Synthetic Peptide of Oncostatin M Reacts in ^{125}I-labeled Oncostatin M in Radioimmune Precipitations

a) *Peptide Synthesis:* The peptide corresponds to residues 6–19 of the Oncostatin M protein and was synthesized by solid phase techniques on a Beckman automated instrument as described (Gentry *et al., J. Biol. Chem.* (1983) *258*: 11219). The peptide was cleaved from the resin using the "low-high" HF procedure (Tam *et al., J. Amer. Chem. Soc.* (1983) *105*: 6442–6445). Purification was accomplished by preparative HPLC.

b) *Production of Antibodies*: The peptide was coupled to bovine γ-globulin as described (Gentry and Lawton, *Virology* (1986) *152*: 421–431). New Zealand white rabbits were primed and boosted (5 times) at 4 sites subcutaneously as described (Gentry and Lawton, *Virology* (1986) *152*: 421–431). Antisera used were obtained 2 weeks after the fifth boost.

c) *Iodination of Oncostatin M*: A sample of partially purified Oncostatin M was radiolabeled with iodine-125 using published procedures (Linsley *et al., PNAS* (1985) *82*: 356–360). An aliquot of the labeled preparation containing 100,000 cpm was mixed with rabbit antiserum directed against the N-terminal 17 amino acids of Oncostatin M (final dilution of 1:20), in the absence or presence of the N-terminal peptide (the N-terminal 17 amino acids of Oncostatin M) (2 μg) and subjected to immune precipitation analysis as described (Linsley *et al., Biochemistry* (1986) *25*: 2978–2986.

Specifically, one tube containing 5 μl was preincubated with 2 μg of the N-terminal peptide in 10 M1 TNEN (20mM Tris pH 7.5, 5mM EDTA, 150mM NaCl, 0.05% Nonidet P-40) containing 0.1% BSA for 30 minutes at 4°C prior to the addition of I^{125} Oncostatin M in 85λ TNEN containing 0.1% BSA and 40 mM dithiothreitol (DTT). Seven tubes containing 5 μl antisera were incubated with I^{125} Oncostatin M in 85 μl TNEN containing 0.1% BSA and 40 mM DTT for 30 minutes at 4°C prior to the addition of 50 μl of 10% formalin-fixed *Staphylococcus aureus* (Pansorbin, Calbiochem).

Following an additional incubation for 30 min. at 4°C, the tubes were microfuged and the pellets were washed 4 times with 1 ml TNEN prior to subjecting them to PAGE analysis. A diffuse band of Mr=32 kD was observed after SDS/PAGE analysis of the immune precipitates. Precipitation of this species was inhibited by the inclusion of an excess of unlabeled peptide corresponding to the N-terminal 17 amino acids of Oncostatin M, indicating that the precipitation was specific for this peptide.

The carbohydrate composition of Oncostatin M was examined by testing for glycosidase sensitivity. Immune precipitates prepared as in c above were treated with buffer, endoglycosidase H, or neuraminidase, as described by Linsley *et al.* (1986). Treatment with endoglycosidase H, an enzyme with specificity for N-linked high mannose oligosaccharides resulted in the appearance of a lower molecular weight species of Mr=24 kD. Only a portion of the radiolabeled material was sensitive to this enzyme, indicating that not all molecules contained high mannose oligosaccharides. Treatment with neuraminidase resulted in the appearance of a single band of Mr=27 kD, indicating that the heterogeneity in size of untreated ^{125}I-labeled Oncostatin M was due to molecular heterogeneity in sialyation of the glycoprotein core. The results indicated that active preparations of Oncostatin M contained a mixture of high mannose and complex N-linked oligosaccharide side chains.

Oncostatin M Isolated from Normal Human Peripheral Blood Lymphocytes (PBL) Production of a Tumor Cell Growth Inhibitor from PBL's

Leucofractions containing PBL's obtained from the Blood Bank, were diluted 1:1 with phosphate buffered saline, pH 7.4 (PBS). Thirty five ml of diluted blood were underlayed with 10 ml of a solution consisting of 9% Ficoll containing 20% by volume of 50% sodium diatrizoate (final specific gravity of 1.080). The gradients were centrifuged at room temperature for 20 minutes at 850×g. Cells were collected from the gradient interface and washed with PBS. Red blood cells were shock-lysed for 3–4 minutes with 10–20 ml of a solution containing 0.8% ammonium chloride and 0.1% Na_4-EDTA.

Cells were collected from red blood cell lysing solution by centrifugation at 600×g for 10 minutes and resuspended in 10 ml of RPMI 1640 medium (GIBCO) containing 5% fetal bovine serum. Thrombin was added to a final concentration of 0.5 U/ml. The cell suspension was agitated for 5 minutes at 37°C and the platelet aggregate allowed to settle for 5 minutes. The suspended cells were transferred to new tubes, recovered by centrifugation, resuspended in 1 ml fetal bovine serum, and transferred into a column containing 0.5 g of brushed, prewetted nylon wool, type 200 (Fenwal).

The nylon wool column was incubated at 37°C for 60 minutes to allow attachment of monocytes and B lymphocytes. The column was then washed with 3×volumes of RPMI 1640 medium (37°C) containing 5% fetal bovine serum and the eluted nonadherent cells (PBL) col-

lected.

PBL (2×10^6 cells/ml) were cultured at 37°C, 5% CO_2-95% air for 96 hours in RPMI-1640 medium (10.4g/l) containing $FeSO_4 \cdot 7H_2O$ (1 mg/l), $ZnSO_4 \cdot 7H_2O$ (2 mg/l) $Na_2SeO_3 \cdot 5H_2O$ (0.017 mg/l), 1-aminoethanol (1 mg/l) human transferrin (5 mg/l), bovine serum albuminlinoleic acid conjugate (Sigma) (200 mg/l), L-glutamine (300 mg/l), penicillin/Streptomycin (100,000 units/l) and phytohemagglutinin-P (Wellcome) (2 mg/l). The supernatants were collected, centrifuged to remove the cells, concentrated by ultrafiltration (Amicon Diaflo membrane YM-10, 10 Kd cut-off), and dialyzed against 0.1 M acetic acid (Spectrapore 3 dialysis tubing). The clarified retentate was lyophilized.

Gel Permeation Chromatography

The crude fraction was reconstituted in 20 ml of 1 M acetic acid (50 mg/ml) and applied to a BioGel P-100 column (2.6×88 cm) equilibrated with 1 M acetic acid at a flow rate of 0.5 ml/min. Twelve ml fractions were collected. An aliquot from each fraction was evaporated and assayed in triplicate for growth inhibitory activity (GIA) of A375 cells. The active fractions were pooled, lyophilized, and rechromatographed on a Bio-Sil TSK-250 column (600×21.5 mm), as described.

Reverse Phase High Pressure Chromatography of TSK-250 Fractions:

The final purification of pooled TSK-250 fractions was achieved by reverse phase HPLC essentially as described. PBL-derived tumor cell inhibitor eluted from μBondapak C18 support at 40–41% acetonitrile and at 26.5% *n*-propanol concentrations, respectively.

Cell Growth Modulatory Assay Using ^{125}I-Iododeoxyuridine Incorporation into DNA (GIA):

These assays were performed in flat-bottomed 96-well tissue culture trays (Costar 3596). Human melanoma cells, A375 (4×10^3), in 50 μl of test sample was added to each well and indubated for 3 days at 37°C. Cells were labeled for 24 hours with ^{125}I-IdU (0.05 μCi/well) and incubated an additional 24 hours. Cells were washed three times, harvested with a multiple sample harvester, and radioactivity was counted in a gamma counter.

Results:

One preparation of PBL-derived tumor cell inhibitor was subjected to automated repetitive Edman degradation. The aminoterminal amino acid sequence is as follows:

```
1         5         10        15
A-A-I-G-X-X-X-K-E-Y-X-V-L-X-X-Q-L-Q-K
```

X represents an amino acid that has not been identified.

A comparison of this sequence with that of U937 factor clearly indicates indentity with the N-terminus of PBL-derived factor.

```
                1         5         10        15
PBL-factor      A-A-I-G-X-X-X-K-E-Y-X-V-L-X-X-Q-L-Q-K
U937-factor     A-A-I-G-S-C-S-K-E-Y-R-V-L-L-G-Q-L-Q-K
```

In the next study, the ability of PBL derived Oncostatin M to effect replication of a variety of cells was investigated. It was found that mouse L929 cells were insenstive to PBL-derived Oncostatin M using up to 1000 GIA units/ml. Human fibroblasts, WI26, were stimulated to grow by treatment with 1000 GIA units/ml. Normal human T-lymphocyte proliferation at 72 hours post mitogenesis was not affected by up to 500 GIA units/ml.

It is evident from the above results, that a novel polypeptide and polypeptide fragments are provided, which can be used for modulating cellular growth. The compound is found to have varying activity depending upon the nature of the cell line involved, so that it may be used by itself or in conjunction with other compounds in modulating cellular growth. The subject polypeptides therefore add an additional polypeptide which may be used with mixtures of cells, both *in vivo* and *in vitro*, to selectively reduce or enhance cellular proliferation of a particular type of cell.

In particular, the factor can be used to treat cells for autologous bone marrow transplants. Use of the factor inhibits the growth of tumor cells in the marrow and may stimulate colony cell formation. Oncostatin M may also be used to stimulate growth of epithelial cells thereby promoting wound healing. In addition, the intact polypeptide or fragments thereof can be used as immunogens to induce antibody formation. The induced antibodies find use to titer Oncostatin M present in a bodily fluid or to modulate the activity of the factor by binding to it. Further, those antibodies together with purified Oncostatin M or fragments thereof serve as a component of diagnostic kits in conjunction with other reagents, particularly antibodies to detect and quantitate Oncostatin M.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

CLAIMS

1. A novel poly(amino acid) of at least eight amino acids that is immunologically cross-reactive with a compound, which compound is a polypeptide capable of being obtained from leukocytes, capable of inhibiting proliferation of neoplastic cells, stimulating proliferation of normal human fibroblasts, not inhibiting human proliferative and cytotoxic T cell responses and not inhibiting granulocytic/myelocytic bone marrow colony cell formation, having a molecular weight in the range of about 17 to 19 kD as determined by gel exclusion chromatography and about 28 kD as determined by SDS-PAGE, and being relatively insensitive to moderate acid and base and moderately elevated temperatures.

2. A poly(amino acid) of Claim 1 containing an amino acid sequence having at least ten consecutive amino acids that correspond to an amino acid sequence depicted in Fig. 1 and differing from said sequence by no more than three amino acids.

3. A poly(amino acid) of Claim 2 containing 12 consecutive amino acids that correspond to an amino acid sequence depicted in Fig. 1 and differing from said sequence by no more than one amino acid, said difference being either a deletion or a conservative substitution.

4. A novel polypeptide composition capable of being obtained from leukocytes, containing a component: Capable of inhibiting proliferation of neoplastic cells, stimulating proliferation of normal human fibroblasts, not inhibiting human proliferative and cytotoxic T cell responses and not inhibiting granulocytic/myelocytic bone marrow colony cell formation, having a molecular weight in the range of about 17 to 19 kD as determined by gel exclusion chromatography and about 28 kD as determined by SDS-PAGE, and being relatively insensitive to moderate acid and base and moderately elevated temperatures; and at a purity providing a specific activity of at least about 10 GIA units/ng protein.

5. A polypeptide composition of Claim 4 wherein said leukocytes are mitogen-activated normal human peripheral blood lymphocytes.

6. A polypeptide composition according to Claim 4, wherein said leukocytes are phorbol diester-induced human histocytic lymphoma cells.

7. A polypeptide composition according to Claim 4, substantially free of cellular components.

8. A polypeptide obtained from a composition according to Claim 4, wherein the specific activity of said polypeptide is at least about 100 GIA units/ng protein.

9. A polypeptide substantially free of cellular debris and other leukocytic proteins, said polypeptide being capable of inhibiting proliferation of neoplastic cells, stimulating proliferation of normal human fibroblasts, not inhibiting human proliferative and cytotoxic T cell responses and not inhibiting granulocytic/myelocytic bone marrow colony cell formation, having a molecular weight in the range of about 17 to 19 kD as determined by gel exclusion chromatography and about 28 kD as determined by SDS-PAGE, and being relatively insensitive to moderate acid and base and moderately elevated temperatures and including amino acid sequences corresponding to the sequences depicted in Fig. 1 with no more than three amino acid differences between the depicted sequence and said corresponding sequence.

10. The polypeptide of Claim 9 wherein said corresponding sequence differs by no more than one amino acid from said depicted sequence.

11. A method for inhibiting the proliferation of neoplastic cells, which comprises contacting said cells with a proliferation inhibiting amount of a composition according to Claim 8.

12. A method for enhancing wound healing comprising contacting a wound with a fibroblast proliferation-stimulating amount of a polypeptide of Claim 8.

13. Antibodies specific for a polypeptide composition according to Claim 1 or Claim 4.

14. Monoclonal antibodies according to Claim 13.

15. A method for detecting the presence of a polypeptide composition according to Claim 4, which comprises:

combining a sample suspected of containing said composition with an antibody according to Claim 13; and

determining the amount of complex formation with said antibody.

16. A DNA sequence encoding a polypeptide according to Claim 8.

17. A method for producing a polypeptide according to Claim 8, which comprises:

growing a cell culture containing cells having an expression construct comprising a DNA sequence encoding a polypeptide according to Claim 4 under the regulatory control of transcriptional and translational regulatory signals functional in said cells, whereby said polypeptide is expressed; and

isolating said polypeptide substantially free of cellular material.

18. A diagnostic kit comprising an antibody of Claim 13 and at least one of a poly(amino acid) of Claim 1 or a polypeptide of Claim 8.

19. A poly(amino acid) of at least eight amino acids substantially as hereinbefore described with particular reference to the Experimental material.

20. A polypeptide or composition containing same substantially as hereinbefore described with particular reference to the Experimental material.

21. An antibody, particularly a monoclonal antibody, substantially as hereinbefore specifically described with particular reference to the Experimental material.

22. A DNA sequence encoding a polypeptide according to this invention.

23. A method according to claim 15 for detecting the presence of a polypeptide substantially as hereinbefore described.

24. Use of a poly(amino acid) or a polypeptide according to any one of claims 1, 4 or 9 for the preparation of a modifier of cellular growth.

CLAIMS

Amendments to the claims have been filed, and have the following effect:–

New or textually amended claims have been filed as follows:–

25. A method of producing a novel poly(amino acid) of at least eight amino acids that is immunologically cross-reactive with a compound, which compound is a polypeptide capable of being obtained from leukocytes, capable of inhibiting proliferation of neoplastic cells, stimulating proliferation of normal human fibroblasts, not inhibiting human proliferation and cytotoxic T cell responses and not inhibiting granulocytic/myelocytic bone marrow colony cell formation, having a molecular weight in the range of about 17 to 19 kD as determined by gel exclusion chromatography and about 28 kD as determined by SDS-PAGE, and being relatively insensitive to moderate acid and base and moderately elevated temperatures which comprises:

growing a cell culture containing cells having an expression construct comprising a DNA sequence encoding said poly(amino acid) under the regulatory control of transcriptional and translational regulatory signals functional in said cells, whereby said poly(amino acid) is expressed; and

isolating said poly(amino acid) substantially free of cellular material.

26. The method of Claim 25, wherein the poly(amino acid) contains an amino acid sequence having at least ten consecutive amino acids that correspond to an amino sequence depicted in Fig. 1 and differing from said sequence by no more than three amino acids.

27. The method of claim 25, wherein the poly(amino acid) contains 12 consecutive amino acids that correspond to an amino acid sequence depicted in Fig. 1 and differing from said sequence by no more than one amino acid, said difference being either a deletion or a consecutive substitution.

28. A method of producing a novel poly(amino acid) substantially as hereinbefore described with particular reference to the examples.

Printed for Her Majesty's Stationery Office by Burgess & Son (Abingdon) Ltd, Dd 8991685, 1987.
Published at The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.