Title: A NOVEL PROTEIN, NEUROLEUKIN

Abstract

A novel protein, neuroleukin, which is capable of extending the survival of in vitro cultured spinal neurons and sensory neurons. The protein is further characterized by the ability to activate immunoglobulin secretion by peripheral blood lymphocytes.
### FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

<table>
<thead>
<tr>
<th>AT</th>
<th>Austria</th>
</tr>
</thead>
<tbody>
<tr>
<td>AU</td>
<td>Australia</td>
</tr>
<tr>
<td>BB</td>
<td>Barbados</td>
</tr>
<tr>
<td>BE</td>
<td>Belgium</td>
</tr>
<tr>
<td>BG</td>
<td>Bulgaria</td>
</tr>
<tr>
<td>BJ</td>
<td>Benin</td>
</tr>
<tr>
<td>BR</td>
<td>Brazil</td>
</tr>
<tr>
<td>CF</td>
<td>Central African Republic</td>
</tr>
<tr>
<td>CG</td>
<td>Congo</td>
</tr>
<tr>
<td>CH</td>
<td>Switzerland</td>
</tr>
<tr>
<td>CM</td>
<td>Cameroon</td>
</tr>
<tr>
<td>DE</td>
<td>Germany, Federal Republic of</td>
</tr>
<tr>
<td>DK</td>
<td>Denmark</td>
</tr>
<tr>
<td>FI</td>
<td>Finland</td>
</tr>
<tr>
<td>FR</td>
<td>France</td>
</tr>
<tr>
<td>GA</td>
<td>Gabon</td>
</tr>
<tr>
<td>GB</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>HU</td>
<td>Hungary</td>
</tr>
<tr>
<td>IT</td>
<td>Italy</td>
</tr>
<tr>
<td>JP</td>
<td>Japan</td>
</tr>
<tr>
<td>KP</td>
<td>Democratic People's Republic of Korea</td>
</tr>
<tr>
<td>KR</td>
<td>Republic of Korea</td>
</tr>
<tr>
<td>LI</td>
<td>Liechtenstein</td>
</tr>
<tr>
<td>LK</td>
<td>Sri Lanka</td>
</tr>
<tr>
<td>LU</td>
<td>Luxembourg</td>
</tr>
<tr>
<td>MC</td>
<td>Monaco</td>
</tr>
<tr>
<td>MG</td>
<td>Madagascar</td>
</tr>
<tr>
<td>ML</td>
<td>Mali</td>
</tr>
<tr>
<td>MR</td>
<td>Mauritania</td>
</tr>
<tr>
<td>MW</td>
<td>Malawi</td>
</tr>
<tr>
<td>NL</td>
<td>Netherlands</td>
</tr>
<tr>
<td>NO</td>
<td>Norway</td>
</tr>
<tr>
<td>RO</td>
<td>Romania</td>
</tr>
<tr>
<td>SD</td>
<td>Sudan</td>
</tr>
<tr>
<td>SE</td>
<td>Sweden</td>
</tr>
<tr>
<td>SN</td>
<td>Senegal</td>
</tr>
<tr>
<td>SU</td>
<td>Soviet Union</td>
</tr>
<tr>
<td>TD</td>
<td>Chad</td>
</tr>
<tr>
<td>TG</td>
<td>Togo</td>
</tr>
<tr>
<td>US</td>
<td>United States of America</td>
</tr>
</tbody>
</table>
A NOVEL PROTEIN, NEUROLEUKIN

The present invention relates to a novel protein which we call neuroleukin. Among biological properties of this material is its ability to extend the survival of sensory ganglia, brain cells and spinal neurons in culture. This protein also may be useful in the diagnosis and treatment of certain diseases, especially involving the immune system.

Unlike most polypeptide growth factors, neuroleukin consists of a single polypeptide chain. It has an apparent molecular weight of approximately 56,000 ± 2000 daltons as determined by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). Its isoelectric point is about pH 8.5 ± 0.5 as determined by isoelectric focusing. Neuroleukin has an amino acid sequence as shown in Tables I and II below. It displays a bioactivity in a neurotrophic assay for maintaining one-half maximal survival of cultured spinal neurons or sensory neurons. Neuroleukin also displays ability to stimulate immunoglobulin secretion by peripheral blood mononuclear cells.

We originally derived neuroleukin from salivary gland and muscle; however, its tissue distribution is widespread. We have also detected it in, inter alia, skeletal tissue, brain, heart, kidney, liver and serum, bone marrow, and human cell lines of lymphoma-leukemia tumor origin. Neuroleukin has demonstrated the ability to maintain the survival of subpopulations of spinal neurons and dorsal root ganglion sensory neurons in vitro. This factor is also apparently involved in spinal motor neuron growth and regeneration in vivo. We have previously reported its presence in other tissue, absent however a method for its
(1984)]. The molecular cloning and expression of neuroleukin,
its activity as a neurotrophic factor for spinal and sensory
neurons and its ability to stimulate immunoglobulin secretion
by cultured peripheral blood mononuclear cells have also
been reported. [M. E. Gurney et al Science 234: 566-574
(1986) and M. E. Gurney et al Science 234: 574-581 (1985)]

Another aspect of the present invention is a process
for producing neuroleukin. The method involves culturing
cells transformed with a vector containing one of several
nucleotide sequences identified as encoding a neuroleukin
polypeptide. The sequence is inserted in the vector under
the control of an appropriate regulatory (e.g. promoter)
sequence. The regulatory sequence may be selected from a
variety of well-known, published sequences. Such selection
is well within the abilities of one skilled in the art of
protein expression. The particular sequences selected
would naturally depend upon the host cells selected for the
ultimate expression system e.g., mammalian cells, bacterial
cells, yeast cells and viruses. Preparation of many such
expression systems has been amply described in the scientific
literature and is well within the skill of the art.

The neuroleukin coding sequence can encompass the two
specific DNA sequences identified herein, as well as sequences
which are capable of hybridizing to the identified sequences
and which, on expression, code for polypeptides which demon-
strate neuroleukin bioactivity. Also included herein are
DNA sequences which differ from those of Tables I and II
below due to the degeneracy of the genetic code (i.e. more
than one codon can code for the same amino acid). The
resulting neuroleukin protein can be characterized by the
amino acid sequences specifically identified below, and
analogs thereof.

Slight variations in the sequences of Tables I and II
which are caused by point mutations, naturally occurring allelic genes and induced modifications should not change the functional protein for which the sequence code on expression. Such variations may be expected to enhance the activity or production of neuroleukin. Such modifications to the sequences, including those due to the degeneracies of the genetic code, are encompassed in the invention. Nucleotide modifications can be deliberately engineered into the DNA sequences employed in this method, which modifications can be made by one skilled in the art using known techniques. Such modifications can include the deletion, insertion or substitution of amino acids. Mutagenic techniques for such replacement or deletion are well known to one skilled in the art.

The biologically active neuroleukin produced by the expression of the neuroleukin sequence in accordance with the present invention can be used as a culture additive in a manner similar to other known nerve growth factors which are commonly employed to extend the survival of various neural cells in vitro. [See, e.g., P.I. Baccaglini et al., Proc. Natl. Acad. Sci. USA, 80: 594-598 (1983)]. Unlike other presently available nerve growth factors, however, neuroleukin is capable of prolonging the viability of sensory ganglia, spinal neurons, and brain cells in vitro. Thus, neuroleukin fills a need in the field of tissue culture additives for extending the life of these neuronal cells. As with other presently available nerve growth factors, the amount of neuroleukin added to a culture medium can be easily experimentally determined, and will depend on the amounts and kinds of other medium supplements, the number and types of tissue cells to be cultured and the size of the culture. Such determinations are simple and within the abilities of one skilled in the art.

The protein of the present invention may be further characterized by its ability to stimulate immunoglobulin
secretion by peripheral blood lymphocytes in a lymphokine assay. It may therefore also be useful as an assay reagent in studies of B-cell function because it appears to mimic the effect of pokeweed mitogen to elicit B-cell differentiation. Thus, neuroleukin may supplement media for, e.g., the culturing of bone marrow cells. Again the amount of neuroleukin added to the media will depend on such factors enumerated above and will involve simple experimentation well within the skill of the art.

As yet a further characteristic of this novel protein growth factor, we have discovered that a portion of the DNA sequence of neuroleukin has significant sequence homology to a portion of the HTLV III/LAV envelope (env) protein gene. The retrovirus HTLV-III/LAV is the causative agent of acquired immune deficiency syndrome (AIDS). Retroviruses of the HTLV/LAV-type are now known as the human immuno-deficiency virus (HIV). A computerized search by the National Biomedical Service, Washington, D.C., illustrated that four published HTLV III env protein sequences scored the most homologous to our novel neuroleukin sequence. Specifically the region of the neuroleukin sequence in Table II extending from codon 403 to codon 447 is significantly homologous to the HTLV III/LAV/ARC sequence from nucleotide 6514 to 6648 as set out in M. Muesing et al., Nature, 313:450-458 (1985). The homologous portion of the HTLV III sequence is in the GP120 protein portion of the env protein. We have preliminarily determined that viral preparations including GP120 inhibit the bioactivity of neuroleukin.

Such homology indicates that neuroleukin or a portion of the neuroleukin sequence may have utility as an in vivo therapeutic treatment for, or as a vaccine against, AIDS. More generally, it may have utility as a treatment for patients infected with HIV. A pharmaceutical formulation of the present invention for use as a therapeutic treatment
or vaccine, will generally comprise active neuroleukin as
above described, together with one or more pharmaceutically
acceptable carriers therefore and optionally other therapeutic
ingredients. The amount of active ingredient will, of
course, depend upon the severity of the condition being
treated, the route of administration chosen, and the specific
activity of the active neuroleukin. The active neuroleukin
may be systematically administered by any route appropriate,
such as parenterally, i.e. by direct injection into the
bloodstream. Dosage of the neuroleukin would be determined
by a physician and vary according to the stage of the disease,
age, physical condition, time and mode of treatment.

Neuroleukin was originally purified from mouse salivary
gland as detailed by the following Examples 1 through 3. However, recombinant methods employing selected expression
vector systems into which the cDNA sequence of Table I or II
below may be conveniently inserted and expressed can provide
a more efficient route of neuroleukin production. Any
number of known expression systems can be conveniently
employed to express the neuroleukin protein coding sequence
of the present invention. An exemplary mammalian expression
system is described in Example 4 below. Techniques for
constructing such expression systems are well-known and
plentifully published, e.g., providing restriction endo-
nuclease enzyme linkers onto the termini of the coding
sequence and inserting the sequence into a vector under
operative association with an expression control sequence
(i.e., promoter/regulator or appropriate host cell signal
sequences). Such manipulations, linkers and expression
sequences are known to those skilled in the art.

The following examples are illustrative only and
therefore are not considered to limit the scope of the
invention.
EXAMPLE 1

Purification of Neuroleukin from Murine Salivary Gland

Approximately 800 ug of neuroleukin was purified from 200 salivary glands obtained from male BALB/c mice (retired breeders). The glands were homogenized in a buffer containing NaH₂PO₄/EDTA/EGTA/leupeptin/PMSF and the homogenate was clarified by centrifugation. A 100,000 x g supernatant was precipitated with polyethylene glycol (PEG), passed over a dye- ligand matrix affinity column (Red agarose, Amicon), and the unbound material was re-precipitated with PEG. Chromatography over Procion Red HE3B-agarose resulted in recovery of the protein in the column flowthrough. This step required dye loading of the agarose according to the procedures of Lowe and Pearson, "Affinity Chromatography on Immobilized Dyes" in Methods in Enzymology, 104: 97-113 (1984).

A second PEG precipitation was followed by gel filtration over AcA54 (LKB, Inc.). The factor eluted from AcA54 with an apparent molecular weight of 55-60 kd. Following gel filtration, the preparation was chromatographed over hydroxylapatite (Biogel HT, BioRad) eluted with a gradient of sodium phosphate, and finally chromatographed over a quarternary ammonium anion exchange HPLC Column (Q300, Synchrom, Inc.) from which it eluted isocratically in 5mM sodium phosphate (pH 7.5). No binding to a cation exchange HPLC column (CM103, Synchrom, Inc.) was observed at this pH and ionic strength. Neuroleukin behaved as an homogeneous species in each chromatographic fractionation.

Purified neuroleukin focuses as a sharp band on isoelectric focusing at about pH 8.5. Thus, it is a monomeric, weakly basic protein. The protein was purified further by chromatography over a C-18 Vydac reverse phase HPLC column (Separations Group) using a 0-95% acetonitrile gradient in 0.1% trifluoroacetic acid (TFA) over 100 min.
Neuroleukin eluted in the gradient at approximately 60% acetonitrile.

The sequence of neuroleukin was determined for both the mouse and human proteins as follows.

**EXAMPLE 2**

Sequence Analysis of Murine Neuroleukin

To obtain peptide fragments from the 56kd factor for sequence analysis, 200µg of the purified neuroleukin was reduced with dithiothreitol, alkylated with iodoacetamide, and then digested to completion with TPCK-treated trypsin (Worthington) (2% w/w/enzyme/substrate) for 18 hr at 37°C. The tryptic digest then was subjected to reverse-phase HPLC using the conditions described above, and the absorbance at both 280 nm and 214 nm was monitored on-line. The well separated peaks indicated on the chromatogram of the tryptic peptides were evaporated to near dryness and subjected directly to N-terminal sequence analysis.

Sequences were determined for eleven different peptides obtained from the mouse neuroleukin. A particularly long sequence, designated T-36 was chosen for the synthesis of oligonucleotide probes. A 33 mer was prepared from the T-36 sequence (5'd CTCCATGTACCCCTGCTGGAAGTAGGCAGAAAA) using an Applied Biosystem Model 380A DNA synthesizer.

(NEN).

Approximately 100,000 phage were screened and eleven independent phage were found to hybridize to the probe, five of which were found to contain inserts of roughly equivalent size. DNA from each of the five phage was digested with EcoRI and subcloned into M13 for DNA sequence analysis using the dideoxy chain termination method [See, e.g. Sanger et al, Proc. Natl. Acad. Sci. U.S.A., 74: 5463-5467 (1977)]. The sequence of two clones (designated C-2 and C-19) was determined completely and revealed a single open reading frame which precisely codes for all of the tryptic peptides sequenced from the mouse neuroleukin.

The sequence contains 2,063 nucleotides which terminate in a 3' poly A+ tract. The length of the cDNA agrees well with the length estimated for the message represented by these clones. Primer extension with the oligonucleotide 33 mer from the 5' end of the message failed to reveal significant extension of the sequence in the 5' direction. Although the long open reading frame encoding the protein extends to the 5' end of the sequence, the first ATG in this reading frame is at nucleotide 50 and is embedded in a canonical sequence for eukaryotic translation initiation sites (CCA/GCCAUG(G)) [See M. Kozak, Nucl. Acids Res., 12:857-872 (1984)]. We presently believe that translation initiates at this codon. From the ATG at nucleotide 50, the open reading frame continues until terminated by a stop codon beginning at nucleotide 1,750.

The first sequence in the cDNA established by peptide data is at codon 13 and the other peptide sequences occur throughout the sequence until the last is reached at codons 498-503. Thus, the established protein sequence begins very near the amino terminal of the deduced amino acid sequence and extends to within 46 amino acids of the carboxy terminal of the deduced sequence. Three potential N-linked glycosylation sites (Arg-X-Thr or Arg-X-Ser) are predicted
by the deduced amino acid sequence, however, no biochemical evidence indicating that glycosylation of the factor occurs has been obtained. The sequence encodes a protein of 558 amino acids. The complete DNA and amino acid sequence of the murine neuroleukin is shown in Table I below.
<table>
<thead>
<tr>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAA TGG ACC CAG TOG CAG CTG CAG TGG GAC GCC ACC GCG ACG TCT</td>
<td>67</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTC ACC CGG AAC CAG TCG TTC CAG AAG CTC GAG TGG CAC GCC GCG AAC TCT</td>
<td>82</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu Thr Arg Asn Pro Glu Phe Gln Lys Leu Leu Glu Thr His Arg Ala Asn Ser</td>
<td>97</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCC ACC CTG AAG CTG CCC GAA CTG TTT GAG CGG GAT CGG GAG GCC TTC AAG AAC Ala Asn Leu Lys Leu Arg Glu Leu Phe Glu Ala Asp Pro Glu Arg Phe Asn</td>
<td>112</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TTC ACC TTG AAG CTC AAC ACC AAC CAT GGG ATT CTG GTG GAC TAC TCC AAG</td>
<td>127</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phe Ser Leu Asn Leu Asn Thr Arg Asn His Gly His Ile Leu Val Asp Tyr Ser Lys</td>
<td>142</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAC CTG GAG AAC AAG GAG GTG CAG AAT GCG GTG GAG GTG GCC AAG TCC AGA</td>
<td>157</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asn Leu Val Asn Lys Glu Val Val MET Glu MET Leu Val Glu Leu Ala Lys Ser Arg</td>
<td>172</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCC GTG GAG CCG GAA CGC AAC AAT TGC AAT GGT TAC AAT AAC TAC ACC</td>
<td>187</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly Val Glu Ala Ala Arg Asp Arg Met Phe Ser Gly Ser Lys Ile Asn Thr Tyr Thr</td>
<td>202</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAG GTG GAC GCG AAA GAT GTG ATG CGG GAG GTG AAC AAG GTT CTG GAC AAG ATG</td>
<td>217</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu Asp Arg Ala Val Leu His Val Ala Leu Arg Asn Arg Ser Asn Thr Pro Ile</td>
<td>232</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAG GTG GAC GCC AAA GAT GTG ATG CGG GAG GTG AAC AAG GTT CTG GAC AAG ATG</td>
<td>247</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys Val Asp Gly Lys Asp Val Val MET Pro Glu Val Arg Arg Asp Lys MET</td>
<td>262</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAG TCT TCC TGC CAG GCG GTG CGG AGT GTG GAC TGG AAA GGG TAC ACT GCC AAA</td>
<td>277</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys Ser Phe Cys Glu Arg Val Arg Ser Gly Asp Trp Lys Gly Tyr Thr Gly Lys</td>
<td>292</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCC TTC ACG GCC ATC ATC AAT GCC GCC GCC TCT CAG CTG GAA CCG CTC</td>
<td>307</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser Ile Thr Arg Leu Ile Asn Ile Gly Ile Gly Ser Asp Leu Gly Pro Leu</td>
<td>322</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATG GTG ACT GAA GCT CTC AAG CCA TAC TGG AAG GGA GTT CCG GTG TGG TTT</td>
<td>337</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MET Val Thr Glu Ala Leu Lys Pro Tyr Ser Lys Gly Gly Pro Arg Val Phe</td>
<td>352</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GTC TCT ACC ATT GAA ACC CAC ATT GCC AAA ACA CTG GCC ACC TAC TCC</td>
<td>367</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val Ser Asn Ile Asp Gly Thr His Ile Ala Lys Thr Leu Ala Ser Ser Pro</td>
<td>382</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAG ACT TCC CTC TTT ATA ATC GCC TCC AAG ACC TCT ACC ACC CAG GAG ACC ATC</td>
<td>397</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu Thr Ser Leu Phe Ile Ile Ala Ser Lys Thr Phe Thr Thr Glu Thr Ile</td>
<td>412</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAG ACT TCC CTC TTT ATA ATC GCC TCC AAG ACC TCT ACC ACC CAG GAG ACC ATC</td>
<td>427</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu Thr Ser Leu Phe Ile Ile Ala Ser Lys Thr Phe Thr Thr Glu Thr Ile</td>
<td>442</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAG ACT TCC CTC TTT ATA ATC GCC TCC AAG ACC TCT ACC ACC CAG GAG ACC ATC</td>
<td>457</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu Thr Ser Leu Phe Ile Ile Ala Ser Lys Thr Phe Thr Thr Glu Thr Ile</td>
<td>472</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAG ACT TCC CTC TTT ATA ATC GCC TCC AAG ACC TCT ACC ACC CAG GAG ACC ATC</td>
<td>487</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu Thr Ser Leu Phe Ile Ile Ala Ser Lys Thr Phe Thr Thr Glu Thr Ile</td>
<td>502</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAG ACT TCC CTC TTT ATA ATC GCC TCC AAG ACC TCT ACC ACC CAG GAG ACC ATC</td>
<td>517</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu Thr Ser Leu Phe Ile Ile Ala Ser Lys Thr Phe Thr Thr Glu Thr Ile</td>
<td>532</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAG ACT TCC CTC TTT ATA ATC GCC TCC AAG ACC TCT ACC ACC CAG GAG ACC ATC</td>
<td>547</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu Thr Ser Leu Phe Ile Ile Ala Ser Lys Thr Phe Thr Thr Glu Thr Ile</td>
<td>562</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAG ACT TCC CTC TTT ATA ATC GCC TCC AAG ACC TCT ACC ACC CAG GAG ACC ATC</td>
<td>577</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu Thr Ser Leu Phe Ile Ile Ala Ser Lys Thr Phe Thr Thr Glu Thr Ile</td>
<td>592</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAG ACT TCC CTC TTT ATA ATC GCC TCC AAG ACC TCT ACC ACC CAG GAG ACC ATC</td>
<td>607</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu Thr Ser Leu Phe Ile Ile Ala Ser Lys Thr Phe Thr Thr Glu Thr Ile</td>
<td>622</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAG ACT TCC CTC TTT ATA ATC GCC TCC AAG ACC TCT ACC ACC CAG GAG ACC ATC</td>
<td>637</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu Thr Ser Leu Phe Ile Ile Ala Ser Lys Thr Phe Thr Thr Glu Thr Ile</td>
<td>652</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAG ACT TCC CTC TTT ATA ATC GCC TCC AAG ACC TCT ACC ACC CAG GAG ACC ATC</td>
<td>667</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu Thr Ser Leu Phe Ile Ile Ala Ser Lys Thr Phe Thr Thr Glu Thr Ile</td>
<td>682</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAG ACT TCC CTC TTT ATA ATC GCC TCC AAG ACC TCT ACC ACC CAG GAG ACC ATC</td>
<td>697</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu Thr Ser Leu Phe Ile Ile Ala Ser Lys Thr Phe Thr Thr Glu Thr Ile</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ACG AAT GCA GAG ACA GCA AAG GAG TGG TTT CTC GAA GCG GCC AAG GAT CCA TCT
Thr Asn Ala Glu Thr Ala Lys Glu Trp Phe Leu Glu Ala Ala Lys Asp Pro Ser

GCA GTT GCA AAG CAC TTT GTC GCC CTC CTG TCT AAC AAG GCC AAA GTC AAA GAG
Ala Val Ala Lys His Phe Val Ala Leu Ser Thr Asn Thr Ala Lys Val Lys Glu

TTT GAA ATT GAC CCT CAA AAG ATG TTC GAG TTT GTC TGT CAA GTT GCC GGC Phe Gly Ile Asp Pro Glu Asn MET Phe Glu Phe Trp Asp Trp Val Gly Gly Arg

TAT TGG CTC GGG ATT GCA ATT TCC ATT GCT CTC CAT GCA GTT TTT GAC
Tyr Ser Leu Trp Ser Ala Ile Gly Leu Ser Ile Ala Leu His Val Gly Phe Asp

CAC TGC GAG CAG CTG CTG TGG TGG CCC TGG TGG ATG GAC CAG CAC TCC CTC AAG
His Phe Glu Glu Leu Leu Ser Gly Ala His Trp MET Asp Glu His Phe Leu Lys

ACG CCC CTG GAG AAG AAT GCC CCC CCC CTG CCA CTG GCT CTA CTG GCC ATC TGG TAG
Thr Pro Leu Glu Lys Asn Ala Pro Val Leu Ala Leu Leu Gly Ile Trp Tyr

ATC AAC TGC TAC GCC TCC GTG GAG ACC CAC GCC TTG CTG GCA CAT GAC CAG TAC ATG
Ile Asn Cys Tyr Gly Cys Glu Thr His Ala Leu Leu Pro Tyr Asp Glu Tyr MET

CAC GCC TTT GCT GCC TAT TCC CAG CAG GGT GAC ATG GAC TGG CTC ACC AAG GAC AAG TAC
His Arg Phe Ala Ala Tyr Phe Glu Glu Gly Asp MET Glu Ser Asn Lys Tyr

ATC ACC AAG TCC GGC GGC GTG GAG CAC CAG CAC ACC GCC CCC ATG CTC GGG Ile Thr lys Ser Gly Ala Arg Val Asp His Glu Thr Gly Pro Ile Val Trp Gly

GAA CCA GGG ACC AAT GGT CAA CAT GGA TTC TAC CAG CTC ATG CAC CAA GCC ACC
Glu Pro Gly Thr Asn Gly Glu His Ala Phe Tyr Glu Leu Ile His Gly Thr

AAG ATG ATA GGC TTT CTC ATC CCT GGC ACC CAG CAC CCC ATA CAG Iys MET Ile Pro Cys Asp Phe Leu Ile Pro Val Glu Thr Glu His Pro Ile Arg

AAA GGT CCG CAT CAC AAG ATC CTC CTG GCT AAG TAC TTT GGG CAG ACT CAG GCC
Iys Gly Leu His His Lys Ile Leu Leu Ala Asp Phe Leu Ala Glu Thr Glu Ala

CTG ATG AAG GGG AAG TIG CTT GAA GAG GCC AAG AAG GAG CTC CAG GCT GCC GGA
EXAMPLE 3

Production of Human Neuroleukin

To obtain human neuroleukin the 33-mer prepared from the T-36 murine sequence (see Example 2 above) was employed as an oligonucleotide probe.

A human cDNA library was prepared in lambda gt10 using oligo (dT) primed double-stranded cDNA of 2 kb and greater synthesized from human muscle poly A+ mRNA from patients suffering from amyotrophic lateral sclerosis (ALS), according to the method of U. Gubler et al., (1983) supra. Linkers were ligated into the EcoR1 site of the vector described in J. J. Toole et al., (1984) supra. The human muscle cDNA library was screened using a modification of the in situ amplification protocol described originally by S.L.C. Woo et al., (1978) supra. using the T-36 oligonucleotide labeled at the 5' end using polynucleotide kinase (New England Biolabs) and -P-32 ATP (NEN).

Approximately 10^5 phage were screened and independent phage were found to hybridize, 4 of which were found to contain inserts of roughly equivalent size. DNA from each of the 4 phage was digested with EcoR1 and subcloned into M13 for DNA sequence analysis using the dideoxy chain termination method of Sanger et al., (1977) supra. The sequence of one clone (designated H510) was determined completely and revealed a single open reading frame.

The complete DNA and amino acid sequence for human neuroleukin is shown in Table II below. The mouse and human nucleotide sequences of Tables I and II respectively are homologous up to the first AUG at the mouse nucleotide 50. After that point, the sequences diverge, as expected for the 5' untranslated regions. To date, no bioactivity has been detected for the human sequence.
<table>
<thead>
<tr>
<th>14</th>
<th>Table II</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>CTCGAGAGCT CGGCC ATG GCC CTC ACC CGG GAC GCC CAG TTT CAG AAG CIG CAG</td>
</tr>
<tr>
<td>45</td>
<td>MET Ala Ala Leu Thr Arg Asp Pro Glu Phe Glu Lys Leu Glu</td>
</tr>
<tr>
<td>60</td>
<td>CAA TGG TAC CGC GAG CAC CGC TCC GAG CTT GAC CCT AAT CTT GGC CCT GAT GCC</td>
</tr>
<tr>
<td>75</td>
<td>Glu Trp Tyr Arg Glu His Arg Ser Glu Leu Asn Leu Arg Arg Leu Phe Asp Ala</td>
</tr>
<tr>
<td>90</td>
<td>AAG GAC CGC TTC ACC AAC CGC TTC AGC AGT AAC ACC AAC CAT GGG CAT</td>
</tr>
<tr>
<td>105</td>
<td>Asn Iys Asp Arg Phe Asn His Phe Ser Leu Thr Leu Asn Thr Leu Asn His Gly His</td>
</tr>
<tr>
<td>120</td>
<td>ATC CTT CTT TAC ACC TCC ACC AGG GAC GAG GAA CAG CAG GAT AAC CAC AAG CIG</td>
</tr>
<tr>
<td>135</td>
<td>Ile Leu Val Asp Tyr Ser Iys Asn Leu Val Thr Glu Asp Val MET Arg MET Leu</td>
</tr>
<tr>
<td>150</td>
<td>GTG GAC TGG GCC AAG TCC AGG GCC TTT GAC GAG GCC GGC GAG GGG AAG TTT GAA</td>
</tr>
<tr>
<td>165</td>
<td>Val Asp Leu Ala Iys Ser Arg Gly Val Glu Ala Ala Arg Gly Arg MET Phe Asn</td>
</tr>
<tr>
<td>180</td>
<td>GGT GAG AAG ATC ACC TAC ACC GAG GGT GGA GGC GCT TTT CCC CIG CIG GCT CIG CIG CIG</td>
</tr>
<tr>
<td>195</td>
<td>Gly Glu Iys Ile Asn Tyr Arg GAG Glu Arg Ala Val Leu His Val Ala Leu Arg</td>
</tr>
<tr>
<td>210</td>
<td>AAG TTT CTT ACG ACC ACC CTC GAC GCC AAG GAT TTT CAT GCA AAG GGC CTC</td>
</tr>
<tr>
<td>225</td>
<td>Asn Arg Ser Asn Thr Pro Ile Leu Val Asp Gly Lys Asp Val MET Pro Glu Val</td>
</tr>
<tr>
<td>240</td>
<td>AAG GAT CTT CIG GAC AAG TCT TAC ACC AAG GCC CAC ACT ACC AAT GCC ATT GTC</td>
</tr>
<tr>
<td>255</td>
<td>Asn Iys Val Leu Asp Iys MET Iys Ser Phe Cys Glu Val Arg Ser Gly Asp</td>
</tr>
<tr>
<td>270</td>
<td>TGG AAG GGG TAC ACC GAG ACC CTC CAG GCC AAG ACC AAG CTC AAG CTC TTC TCA</td>
</tr>
<tr>
<td>285</td>
<td>Thr Iys Gly Tyr Thr Gly Iys Thr Ile Thr Asp Val Ile Asn Ile Gly Glu Val</td>
</tr>
<tr>
<td>300</td>
<td>GGC TGC CAG CTT GGA GCC ACC TGC AAG GCA TGCC TAA CTC TAC TTC TCA</td>
</tr>
<tr>
<td>315</td>
<td>Gly Ser Asp Leu Gly Pro Arg VAL MET Thr Glu Ala Leu Lys Pro Tyr Ser Ser</td>
</tr>
<tr>
<td>330</td>
<td>GGA GST OCC GCC GTC TGG TAT GTC TCC AAG ATT GAT GGA ACT CAC ATT GGC AAA</td>
</tr>
<tr>
<td>345</td>
<td>Gly Gly Pro Arg VAL Thr Tyr Val Ser Asn Ile Asp Glu Thr His Ala Lys</td>
</tr>
<tr>
<td>360</td>
<td>ACC CIG GCC CAG CTC ACC CAC CGG GAG TCC CTG TTC ACT AAT GCC TCC CAA ACC</td>
</tr>
<tr>
<td>375</td>
<td>Thr Leu Ala Glu Leu Asn Pro Glu Ser Ser Leu Phe Ile Ile Ala Ser Lys Thr</td>
</tr>
<tr>
<td>390</td>
<td>TTT ACT ACC CAG GAG ACC AAT GCA GAG AAG GGC GAG CAG TGG CTT CTC</td>
</tr>
<tr>
<td>405</td>
<td>Phe Thr Thr Glu Thr Ile Thr Asn Ala Glu Thr Ala Lys Glu Trp Phe Leu</td>
</tr>
</tbody>
</table>
CAG GCG CCC AAG GAT OCT TCT GCA GIG GCG AAG CAC TTT GTT GCG CIG TCT ACT
Gln Ala Ala Lys Asp Pro Ser Ala Val Ala Lys His Phe Val Ala Leu Ser Thr

AAC ACA ACC AAA GTG AAG GAG TTT GGA ATT GAC OCT CAA AAC ATG TTC GAG TTC
Asn Thr Thr Lys Val Lys Glu Phe Gly Ile Asp Pro Gln Asn MET Phe Glu Phe

TGG GAT TCG GTG GGA GGA GCC TAC TGG CIG TGG TGG GCC ATC GGA CTC TCC ATT
Trp Asp Trp Val Gly Gly Arg Tyr Ser Leu Trp Ser Ala Ile Gly Leu Ser Ile

GCC CIG CAC GTG GGT TTT GAC AAG TTC GAG CAG CIG CTC TGG GGC GTG CAC TGG
Ala Leu His Val Gly Phe Asp Asn Phe Glu Gln Leu Leu Ser Gly Ala His Trp

ATG GAC CAG CAC TTC GCC ACG ACG CCC CIG GAG AAG AAC GCC CCC GTC TTG CIG
MET Asp Gin His Phe Arg Thr Thr Pro Leu Glu Lys Ala Pro Val Leu Leu

GCG CIG CIG GTG ATC TGG TAC ATC AAG TAC TTC GAT GGG TGG TGG GAT ACA CAC GCC ATG
Ala Leu Leu Gly Ile Trp Tyr Ile Asn Cys Phe Gly Cys Glu Thr His Ala MET

CTG CCC TAT GAC CAG TAC ATG CAC GCC TTT GCT GAG TTC GAG CAC GCC GAC
Leu Pro Tyr Asp Gin Tyr Leu His Arg Phe Ala Ala Tyr Phe Gln Gln Gly Asp

ATG GAG TCC ATT GGG AAA TAC ATC ATT ACC AAA TCT GGA ACC GTG GCC GAC CAC CAG
MET Glu Ser Asn Gly Lys Tyr Ile Thr Lys Ser Gly Thr Arg Val Asp His Gin

ACA GCC CCC ATT GTG TGG GGG GAG CCA GGG ACC AAT GCC CAG CTT ATG TCT
Thr Gly Pro Ile Val Trp Gly Gly Pro Gly Thr Asn Gly Gin His Ala Phe Tyr

GAG CTC ATC CAC GAA GCC ACC AAG ATG ATA CCC TGG GAC TGC TCC ATC CTC CCC GTC
Gln Leu Ile His Gin Gly Thr Lys MET Ile Pro Cys Asp Phe Leu Ile Pro Val

CAG ACC CAG CAC CCC ATA GGG AAG GGT CTG CAT CAC ACG ATC CTC CTT GGC CAA
Gln Thr Gin His Pro Ile Arg Lys Gly Leu His His Lys Ile Leu Ala Asn

TTC TGG GCC CAG ACA GAG GCC CIG ATG AGG GAA TGG ACG GAG GAG GCC CGA
Phe Leu Ala Gin Thr Glu Ala Leu MET Arg Gly Lys Ser Thr Glu Ala Arg

AAG GAG CTC CAG GCT GGC GGC AAG AGT CCA GAG CAC CIT GAG AGG CIG CTA CCA
Lys Glu Leu Gln Ala Ala Gly Iys Ser Pro Glu Asp Leu Glu Arg Leu Leu Pro

1410 1425 1440 1455
CAT AAG GTC TTT GAA GGA AAT GGC ACC ACC AAT TCT ATT GTG TTC GTG CTC His Lys Val Phe Glu Gly Asn Arg Pro Thr Asn Ser Ile Val Phe Thr Iys Leu

1470 1485 1500 1515
ACA CCA TTC ATT GTC GCA CCG TTC GGC TAC GTC GAC AAT TTC GAC AAT TTC Thr Pro Phe MET Leu Gly Ala Ala Val Ala MET Tyr Glu His Iys Ile Phe Val

1530 1545 1560
CAG GCC ATC ATC TGG GAC ATC AAT GAC TGG GCA TTT GAG CAT GAC AAT TTC GGA Gln gly Ile Ile Trp Asp Ile Asn Ser Phe Asp Gln Trp Gly Val Glu Leu Gly

1575 1590 1605 1620
AAG CAG CTT GCT AAG AAA ATA GAG CCT GAG CTT GAC ACT GCT CAA GIG ACC Lys Glu Leu Ala Iys Iys Ile Glu Pro Glu Leu Asp Gly Ser Ala Gln Val Thr

1635 1650 1665
TCT CAC GAC GCT TCT ACC AAT GGG CTC ATC AAT TTC AAT AAT CAG CAG GGC CAG Ser His Asp Ala Ser Thr Asn Gly Leu Ile Asn Thr Iys Lys Glu Leu Gly

1680 1699 1709 1719 1729 1739
GCC AGA GTC CAA TAAACTCGTG CTCATCTGCA GCTCCTCGTG TACACTGCTT TTCTCTCTC Ala Arg Val Gln

1749 1759 1769 1779 1789 1799 1809
GTCCTCTCTC CCCGAGCGG GCACTGCAATG TCTCCTGACA CCAACCAGAG CACCCCTGGG TGGGCGTT

1819 1829 1839 1849 1859 1869 1879
GACCACAGC CCCGCCAGG GGAAGGCTGG TCTCCCCAG CCAACGCCCC AGCCTGCGGA CGTCTATGCT

1889 1899 1909 1919 1929 1939 1949
CCCTCTCTCTG TGGAGGTTGC TGGACGACT TGGAGCACT GACGCTCTGC ACCACACTTC ACCTGTCTCA

CATCCTACTG AAAAAACRA AGATGGCAGG GAGGAGTAA AAAAAAAA AAAAAAAA AAAAAAAA

2029

AAAAAAAAA AAAAA
EXAMPLE 4

Expression of Murine Neuroleukin

The murine neuroleukin sequence identified in Example 2, was inserted into a vector and expressed in mammalian cells as follows: The expression vector p91023(B) described by G. G. Wong et al., Science, 228: 810-815 (1985) vector contains the adenovirus major late promoter, a simian virus 40 (SV40) polyadenylation sequence, an SV40 enhancer and origin of replication and the adenovirus virus-associated gene. The mouse neuroleukin sequence identified in Table I above was inserted into the EcoRI site of the p91023(B) vector downstream of the adenovirus major late promoter. This construct was transfected into COS-1 cells using DEAE-dextran-mediated DNA transfection with the addition of chloroquin treatment as described by R. J. Kaufman et al., Mol. Cell Biol, 2: 1304 (1982). Thereafter an immunoprecipitable 56kd polypeptide was secreted into the culture supernatant. Control cultures transfected with the p91023(B) vector alone did not produce detectable 56kd factor.

EXAMPLE 5

Biological Assay of Murine Neuroleukin

The biological activity of the neuroleukin expressed in Example 4 was assessed using three bioassays — two assays for neurotrophic activity in spinal neurons and in sensory neurons and an assay for lymphokine activity.

A. One neurotrophic assay employs cultured chick spinal neurons which are dissociated from 5 day embryonic chick spinal cord using 0.25% trypsin and cultured at 10,000 cells per 16 mm well on a substrate of poly-ornithine coated with laminin (5 ug/ml, Bethesda Research Labs.). The cells are cultured in L-15 containing 10% zeta Sera-D (processed adult bovine serum, AMF), 6 mg/ml glucose,
100U/ml penicillin and 100 ug/ml streptomycin at 37°C and 5% CO₂ in a humidified incubator. After 24 hrs. in culture, 50% of the neurons plated initially die in the absence of added neuroleukin. Thus, the biological activity of the neuroleukin polypeptide can be quantitated by determining the amount of transfected cell supernatant required to maintain one-half maximal survival of the cultured spinal neurons. The purified neuroleukin maintains one-half maximal survival at a concentration of 1.25 x 10⁻¹¹ M. Neurons are scored microscopically as cells with neurites.

B. To study the effect of neuroleukin on sensory neurons from 10-day and 16-day chick embryo dorsal root ganglia, sensory ganglia are dissected under sterile conditions, incubated in Ca, Mg-free Hank's balanced salt solution (CMF-HBSS) for 20 minutes at 37°C, and digested in 0.10% trypsin in CMF-HBSS for 10 minutes at 37°C. Trypsin-treated ganglia are washed in the culture medium, i.e., Dulbecco's modified Eagle medium (DMEM) with 10% heat-inactivated horse serum (v/v%) and 1.5mg% added glucose, and dissociated into single-cell suspensions by trituration through a glass Pasteur pipette. To enrich for neurons, cell suspensions are preplated on tissue culture plastic for 3 hours at 37°C. Neurons are then seeded at 5,000 cells per 16mm tissue culture well. Each well has been treated with 0.3 ml of 100 ug/ml poly-L-ornithine (hydrobromide, M.W. 30,000-70,000, Sigma) overnight at 4°C followed by 0.3 ml of 10 ug/ml purified laminin (Bethesda Research Labs) for 3 hrs at 37°C. Neuroleukin expressed in Example 4 was added to experimental cultures in the form of serum-free conditioned medium. A control is provided by conditioned medium collected from COS-1 cells transfected with the p91023(B) vector only. Cultures are maintained in a 5% CO₂:95% air, humidified atmosphere at 37°C for 48 hours, at which time they are examined for cells with neurites which are scored as neurons.
Maximal stimulation by neuroleukin supports the survival of approximately 55% of the neurons cultured from 10-day chick embryo dorsal root ganglia. In the control culture medium alone, approximately 15% of the neurons survive for 48 hours. Thus, neuroleukin promotes survival of 40% of the sensory neurons initially plated. On the 48 hour survival of neurons in dissociated cell cultures from 16-day chick embryo dorsal root ganglia, stimulation by neuroleukin also supports the survival of approximately 50% of the neurons cultured.

To demonstrate that neuroleukin is capable of supporting the survival of the responsive neurons in long-term culture, 10-day chick embryo dorsal root ganglion neurons are cultured with the culture medium being changed every three days. After 1 week in culture, a 1:25 dilution of neuroleukin supports the survival of 33% of neurons cultured. In addition, the surviving neurons have extensive neurite outgrowth. In control cultures there are no surviving neurons.

Addition of neuroleukin to cultures of neonatal rat superior cervical ganglion (SCG) neurons had no effect on neuronal survival. Few SCG neurons survive when cultured in medium alone or with added neuroleukin whereas SCG neurons experience a high survival rate when treated with NGF. Thus the biological activity of the murine neuroleukin is distinct from that of previously known neurotrophic factors, e.g., nerve growth factor.

C. To assay for lymphokine activity, freshly drawn peripheral venous blood is obtained from consenting young adults (age <50 yr) known to be high responders to pokeweed mitogen (>1000 ng/ml lg). Mononuclear cells (MNCs) are isolated on a Ficoll-Hypaque gradient by following standard protocols. Cells then are suspended in Hank's Balanced Salt Solution, washed ten times at 4 C, and then resuspended in culture medium at 10^6 cells per ml. The medium is RPMI
1640 supplemented with 10% fetal bovine serum, 4 nmol glutamine and 0.1 ng/l gentamycin. The pokeweed mitogen control is used at a 1:100 final concentration from stock (GIBCO). For assay of neuroleukin stimulated Ig secretion, 0.2 ml of the MNC cell suspension is cultured with neuroleukin expressed in Example 4 above in round-bottom 96 wellplates (Costar) for 7 days at 37°C in a 5% CO2 humidified incubator. Supernatants of quadruplicate cultures then are pooled and assayed by ELISA for Ig content using anti-human IgG (H + L) and biotinylated anti-human (Vectastain) as previously described. Parallel cultures of unseparated MNCs are similarly cultured for 7 days with pokeweed mitogen as a control. Both neuroleukin and pokeweed mitogen produce comparable induction of immunoglobulin secretion. Mock serum-free culture supernatant collected from COS-1 cells that were transfected with the expression vector alone did not contain neuroleukin and did not induce Ig secretion. Neuroleukin dependent Ig induction is apparently both monocyte and T-cell dependent. Removal of monocytes from mononuclear cells reduces Ig production in response to both PWM and neuroleukin. Upon removal of T-cells and monocytes the resultant B-cell subset did not differentiate into Ig-secreting cells when cultured with neuroleukin.

The amount of neuroleukin produced by transfected COS-1 cells can be quantitated by determining a dose-response relationship between the amount of transfected cell supernatant added to the assay well and the content of Ig produced in the assay well. Approximately one biological unit of neuroleukin can be defined as the amount of neuroleukin required to produce one-half maximal stimulation of Ig synthesis.

Fractionation of bulk stimulated MNCs into T-cell and B-cell subsets reveals that neuroleukin is a PWM-induced, T-cell secretory product. Neuroleukin production can be induced in mononuclear cells freshly isolated from peripheral
blood by PWM. MNL's stimulated with PWM are fractionated into subsets of T-cells, B-cells and monocytes to determine whether neuroleukin is a T-cell, B-cell, or monocyte product. In each experiment, PWM-stimulated T-cell subsets produced neuroleukin, whereas the B-cell subset failed to produce neuroleukin.

Numerous modifications and variations in practice of the invention are expected to occur to those skilled in the art upon consideration of the foregoing description. For example, one skilled in the art may construct analogs of the sequences identified above, to produce a related "family" of neuroleukins. Similarly, therapeutic compositions or vaccines for use in treating AIDS may employ only a portion of the sequences identified herein. Such modifications will be encompassed in the scope of the appended claims.
WHAT IS CLAIMED IS:

1. A neuroleukin protein substantially free from association with other naturally occurring proteins characterized by:
   a) a single polypeptide chain;
   b) an amino acid sequence selected from the group consisting of the sequence of Table I and the sequence of Table II; and
   c) an apparent molecular weight of about 56,000 ± 2000 daltons as determined by SDS-PAGE.

2. The protein of Claim 1, further characterized by
   d) the ability to maintain one-half maximal survival of spinal or sensory neurons cultured in vitro at a neuroleukin concentration of approximately 1.25 x 10^{-11} M.

3. The protein according to Claim 1, further characterized by
   e) the ability to activate immunoglobulin secretion by peripheral blood lymphocytes.

4. A method for producing a neuroleukin protein comprising culturing a cell transformed with a vector comprising a DNA sequence selected from the group consisting of
   (a) DNA sequence of Table I
   (b) DNA sequence Table II
   (c) DNA sequences which hybridize to any of the foregoing DNA sequences and which code on expression for a polypeptide that is substantially immunologically equivalent to a neuroleukin polypeptide;
said DNA sequence being operatively linked to a regulatory sequence therefore.

5. A tissue culture medium for culturing neural cells comprising an effective amount of a neuroleukin protein according to Claim 1.

6. A tissue culture medium for culturing bone marrow cells comprising an effective amount of a neuroleukin protein according to Claim 1.

7. A pharmaceutical composition comprising an effective amount of neuroleukin protein.

8. A therapeutic composition for treating patients infected with human immunodeficiency virus comprising an effective amount of the protein of claim 1, 2 or 3.

9. A method for treating a patient infected with human immunodeficiency virus comprising administering an effective amount of the protein of claim 1 or 2 or 3.
INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL4 C07K 15/00; C12P 21/00; C12N 15/00, 5/00, 5/02; A61K 37/02

U.S. CL 530/399, 350, 351; 435/68, 172.1, 172.3, 240, 241; 514/2

II. FIELDS SEARCHED

Minimum Documentation Searched

Classification System

Classification Symbols

U.S. 530/399, 350, 351, 827, 839; 435/68, 172.3, 240, 241, 948;
514/2; 935/60

Documentation Searched other than Minimum Documentation

to the Extent that such Documents are Included in the Fields Searched

III. DOCUMENTS CONSIDERED TO BE RELEVANT

Category

Citation of Document, with indication, where appropriate, of the relevant passages

Relevant to Claim No.

P, X

SCIENCE, VOL. 234, ISSUED OCTOBER 1986,
"NEUROLEUKIN: A LYMPHOKINE PRODUCT OF
LECTIN-STIMULATED T CELLS," (GURNEY),
PGS. 574-81. SEE ENTIRE DOCUMENT.

1-4

P, X

SCIENCE, VOL. 234, ISSUED OCTOBER 1986,
"MOLECULAR CLONING AND EXPRESSION OF
NEUROLEUKIN, A NEUROTROPIC FACTOR FOR
SPINAL AND SENSORY NEURONS," (GURNEY),
PGS. 566-73. SEE ENTIRE DOCUMENT.

1-6

X

NATURE, VOL. 307, ISSUED FEBRUARY 1984,
"SUPPRESSION OF SPROUTING AT THE NEUROMUSCULAR JUNCTION BY IMMUNE SERA," (GURNEY)
PGS. 546-58. SEE ABSTRACT.

5-6

X

NEW ENGLAND JOURNAL OF MEDICINE, VOL. 311,
ISSUED OCTOBER 1984, "INHIBITION OF
TERMINAL AXONAL SPROUTING BY SERUM FROM
PATIENTS WITH MYOTROPHIC LATERAL
SCLEROSIS," (GURNEY), PGS. 933-39. SEE
ENTIRE DOCUMENT.

5-6


* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority data claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

03 AUGUST 1987

Date of Mailing of this International Search Report

26 AUG 1987

International Searching Authority

ISA/US

Signature of Authorized Official

GARNET D. DRAPER
### V. OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claim numbers **because they relate to subject matter not required to be searched by this Authority, namely:**

2. Claim numbers **because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out,** specifically:

### VI.X OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This International Searching Authority found multiple inventions in this international application as follows:

1. CLAIMS 1-3, 7-9, drawn to a protein, pharmaceutical composition, an method for treatment, class 530 and 514, subclasses 399 and 2, respectively.

2. CLAIM 4, drawn to a method of preparing a protein, class 435, subclass 172.3.

3. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

4. Telephone practice. See attachment

5. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

6. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

7. As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- The additional search fees were accompanied by applicant's protest.
- No protest accompanied the payment of additional search fees.

---

Form PCT/ISA/210 (supplemental sheet (2) (May 1986)
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of Document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to Claim No</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>PROC. NATL. ACADEM. SCI., VOL. 80, ISSUED JANUARY 1983, &quot;SOME RAT SENSORY NEURONS IN CULTURE EXPRESS CHARACTERISTICS OF DIFFERENT PAIN SENSORY CELLS,&quot; (BACCAGLINI), PGS. 594-98.</td>
<td>1-3, 6-7</td>
</tr>
<tr>
<td>A</td>
<td>DEVELOPMENTAL BIOLOGY, VOL. 98, ISSUED JULY 1983, &quot;NEURITE EXTENSION BY PERIPHERAL AND CENTRAL NERVOUS SYSTEM NEURONS IN RESPONSE TO SUBSTRATUM-BOUND FIBRONECTIN AND LAMININ&quot;, (ROGERS), PGS. 212-20.</td>
<td>5-6</td>
</tr>
<tr>
<td>A</td>
<td>PROC. NATL. ACADEM. SCI., VOL 82, ISSUED SEPTEMBER 1985, &quot;CLONING, SEQUENCE, AND EXPRESSION OF A HUMAN GRANULOCYTE/MACРОPHAGE COLONY-STIMULATING FACTOR&quot;, (CANTRELL), PGS. 6250-54.</td>
<td>1-4</td>
</tr>
<tr>
<td>A</td>
<td>JOURNAL INVESTIGATIVE DERMATOLOGY, VOL. 85(1), ISSUED 1985, &quot;MOLECULAR CLONING OF HUMAN AND MURINE INTERLEUKIN-2 GENE AND THEIR EXPRESSION IN VARIOUS HOST CELLS, &quot;(FUJITA), PGS. 180S-182S.</td>
<td>1-4</td>
</tr>
<tr>
<td>A</td>
<td>PROC. NATL. ACADEM. SCI., VOL. 81, ISSUED DECEMBER 1984, &quot;NUCLEOTIDE SEQUENCE OF HUMAN MONOCYTE INTERLEUKIN-1 PRECURSOR cDNA,&quot; (AURON), PP 7907-11.</td>
<td>1-4</td>
</tr>
</tbody>
</table>
ATTACHMENT TO FORM PCT/ISA/210, PART VI.

III. Claims 5-6, drawn to a tissue culture, class 435, subclass 240.

Telephone Approval:

A $280.00 payment was approved by Mary Bak on July 27, 1987 for Examiner Garnette D. Draper to search Groups I, II, and III to be charged to Deposit Account Number 071060.

REASONS FOR HOLDING LACK OF UNITY OF INVENTION:

The invention as defined by Group I (claims 1-3 and 7-9) is drawn to a protein, pharmaceutical composition and method of treatment, classified in Class 530 and 514, subclasses 399 and 2 respectively. Group II (claim 4) is drawn to a method of using biogenetics to prepare a protein, classified in Class 435, subclass 172.3. And Group III (claims 5-6) is drawn to a treated tissue culture, classified in Class 435, subclass 240.

PCT Rule 132 permits claims to a product an a (one) use of said product. Claims directed to the tissue culture
represent a separate composition from claims 7-9 of Group I, and the use of the protein to treat immunodeficiencies is separate from the use of the protein to proliferate tissue cultures.

TIME LIMIT FOR FILING A PROTEST

Counsel was advised that they have no right to protest for any group not paid for, and that they have 15 days from the mailing of the search report (Form 210) in which to file a protest. In accordance with PCT Rule 40.2 applicant may protest the holding of lack of unity only with respect to groups paid for.