Description

FIELD OF THE INVENTION

This invention relates to Leukaemia Inhibitory Factor (LIF), to the production of LIF in substantially pure form, and to the cloning and expression of recombinant LIF.

BACKGROUND OF THE INVENTION

Mature blood cells are produced by the clonal proliferation and concomitant differentiation of immature precursor cells (Metcalf,D. (1984) The Hemopoietic Colony Stimulating Factors, Elsevier, Amsterdam.). For normal haemopoietic cells, the two processes of proliferation and differentiation are tightly coupled so that the short-lived mature cells are continually replenished. At least four biochemically distinct growth factors, the colony-stimulating factors (CSFs), have been shown in vitro to stimulate the proliferation and differentiation of precursor cells of granulocytes and macrophages: G-CSF, M-CSF, GM-CSF and Multi-CSF (IL-3) (see Metcalf, D. (1987), Proc.R.Soc.B. 230, 389-423, for review).

In contrast to normal cells, leukaemic myeloid cells are characterized by an uncoupling of proliferation and differentiation so that immature progenitor cells accumulate, retain their proliferative capacity and fail to differentiate. There has been considerable controversy concerning the nature of biological factors that are able to induce the differentiation of myeloid leukaemic cells in vitro and whether certain factors are capable of inducing differentiation in the absence of proliferation. Indeed, it has been proposed that the events of proliferation and differentiation are necessarily mediated by different factors (Sachs, L. (1982), J.Cell.Physiol.Suppl., 1, 151-164). On the one hand, two groups have described and purified an activity (MGI-2 or D-factor) capable of inducing the differentiation of the murine myeloid leukaemic cell line M1, that does not stimulate the proliferation of normal progenitor cells (Lipton, J.H. and Sachs, L. (1981), Biochem.Biophys. Acta. 673, 552-569; Tomida, M., Yamamoto-Yamaguchi, Y., and Hozumi, M. (1984) J.Biol.Chem. 259, 10978-10982). On the other hand, the present inventors have previously shown that one of the CSFs, G-CSF, is a strong differentiation-inducing stimulus for the murine myeloid leukaemic cell line, WEHI-3B D^+, as well as being a proliferative and differentiative stimulus for normal cells (Nicola,N.A., Metcalf, D., Matsumoto, M. and Johnson, G.R. (1983), J.Biol.Chem. 258, 9017-9023). Moreover, Tomida, et.al. (Tomida, M., Yamamoto-Yamaguchi, Y., Hozumi, M., Okabe, T. and Takaka, F. (1986), FEBS Lett., 207, 271-275) have shown that recombinant G-CSF is also able to induce the macrophage differentiation of M1 cells. The relationship between these factors has been a matter of debate. The situation was further complicated by the finding that tumour necrosis factor α(TNFα) is capable of stimulating the differentiation of the human myeloblastic cell line ML-1 (Takeda,K., Iwamoto, S., Sugimoto, H., Takuma,T., Kawatani,N., Noda,M., Masaki,A., Morise,H., Arimura,H. and Konno,K. (1986) Nature 323, 338-340).

In an attempt to resolve the discrepancies between the data of these groups, the present inventors have biochemically fractionated the medium conditioned by Krebs II ascites tumour cells used in a number of previous studies and shown it to contain not only authentic G-CSF and GM-CSF, active on normal progenitor cells as well as WEHI-3B D^+ cells, but also two biochemically distinct, but functionally similar, factors capable of inducing the differentiation of M1 cells. These latter factors have been termed leukaemia inhibitory factor (LIF)-A and LIF-B because of their ability to suppress the proliferation of M1 leukaemic cells in vitro and it has been shown that they do not induce the differentiation of WEHI-3B D^+ cells and do not stimulate the proliferation of normal granulocyte/macrophage progenitor cells.

SUMMARY OF THE INVENTION

LIF, in accordance with the present invention, is defined as a molecule that has the following two properties: (1) it has the ability to suppress the proliferation of myeloid leukaemia cells such as M1 cells, with associated differentiation of the leukaemic cells; and (2) it will compete with a molecule having the defined sequence of murine LIF or human LIF hereein for binding to specific cellular receptors on M1 cells or murine or human macrophages. LIF, has the potential for use as a therapeutic non-proliferative agent for suppressing some forms of myeloid leukaemia as well as a reagent for modifying macrophage function and other responses to infections, and clinical testing and research studies relating to these.

The term "polypeptide having LIF activity" as used herein denotes a polypeptide or glycopolypeptide having the properties of LIF as defined above, including polypeptides having an amino acid sequence which is fully or partially homologous with the amino acid sequence of either murine or human LIF as disclosed herein. This term also includes polypeptides which are fully or partially homologous with a portion only of

the amino acid sequence of murine or human LIF provided that the polypeptide has the properties of LIF as defined above. This term further includes polypeptides or glycopolypeptides produced by expression in a host cell which are inactive when expressed but which are processed by the host cell to yield active molecules, as well as polypeptides or glycopolypeptides which are inactive when expressed but which are selectively cleavable in vitro or in vivo to yield active molecules.

Murine LIF is a molecule having the following biological properties:

(a) induction of macrophage differentiation in cells of the murine myeloid leukaemic cell line M1 with loss of proliferative capacity and death of the clonogenic leukaemic cells, an action potentiated by G-CSF;

(b) selective binding to high affinity receptors on M1 cells and on normal murine monocytes and macrophages from the peritoneal cavity, spleen and bone marrow, with the number of receptors increasing with macrophage maturation or functional activation, but not to granulocytic, erythroid or lymphoid cell from these tissues;

(c) specific binding of ^{125}I-LIF to high affinity receptors is not competed for by G-CSF, GM-CSF, Multi-CSF (interleukin-3), M-CSF, interleukins 1,2, 4 or 6, endotoxin or a variety of other growth factors, but is competed for by unlabeled LIF.

(d) elevation by bacterial endotoxin in the serum of normal or athymic mice, but not in endotoxin-resistant C3H/HeJ mice;

(e) production by various tissues including lung, salivary gland, peritoneal cells and bone shaft;

(f) reduction of the survival time in vitro of normal granulocyte-macrophage progenitor cells when grown in the absence of CSF;;

(g) an inability to suppress proliferation or induce differentiation of WEHI-3B D$^+$ murine myeloid leukaemic cells, murine myeloid cells transformed to leukaemogenicity by infection with retroviruses expressing GM-CSF or Multi-CSF, the murine leukaemic cell lines WEH1265 and WR19;

(h) no ability to stimulate the proliferation of normal progenitor cells of the granulocyte, macrophage, eosinophil, megakaryocyte, erythroid and mast cell lineages, and an inability to suppress the clonal proliferation or alter the quantitative responsiveness to stimulation by CSFs in vitro of progenitors of normal granulocytes, macrophages, megakaryocytes, eosinophils or natural cytotoxic cells or the proliferation of cells of the continuous cell lines 32CD1.13 and FDCP-1;

(i) an inability to compete with the iodinated derivatives of granulocyte colony-stimulating factor (G-CSF) for binding to specific cellular receptors despite the ability of G-CSF to induce differentiation in M1 and WEHI-3B D$^+$ murine myeloid leukaemic cells and to potentiate the action of LIF on M1 cells;

(j) an inability of the action of LIF to be inhibited by antisera specifically raised against tumour necrosis factor (TNF);

(k) transcripts for murine LIF are present constitutively in the cytoplasm of LB3 and E9.D4 T cells, are not induced by the lectin concanavalin A in these cells, and are present in a number of other cell types.

Murine LIF has also been determined to have the following properties:

(l) a single subunit glycoprotein with molecular weight of 58,000± 5,000 as determined by electrophoresis in 8-25% gradient polyacrylamide gels containing sodium dodecyl sulphate with or without reducing agent (2-mercaptoethanol or dithiothreitol), and a molecular weight of 23,000 ± 5,000 after treatment with the endoglycosidase, N-glycanase;

(m) an isoelectric point between 8.6 and 9.3;

(n) the primary amino acid sequence detailed in Figures 10, 11 and 15 herein;

(o) is encoded by the nucleotide sequence detailed in Figures 10, 11 and 15 herein;

(p) a specific activity of 1-2 x 10^8 units/mg (where 50 units are defined as the amount of LIF which in one millilitre induces a 50% reduction in the clone formation by murine M1 myeloid leukaemic cells).

(q) is encoded by a unique gene on murine chromosome 11 (as determined by analysis of a panel of mouse/Chinese hamster ovary somatic cell hybrids) bounded by restriction endonuclease cleavage sites as illustrated in Figure 19 herein;

(r) is homologous to a human gene sequence bounded in chromosomal DNA by restriction endonuclease cleavage sites as illustrated in Figure 27 herein.

Human LIF is a molecule having the following biological properties of the native and or yeast derived recombinant product:

(a) induction of macrophage differentiation in cells of the murine myeloid leukaemia cell line M1 with loss of proliferative capacity and death of the clonogenic leukaemia cells;

(b) no ability to stimulate the proliferation of normal human progenitor cells of the granulocyte macrophage, eosinophil and erythroid lineages;

(c) an ability in combination with G-CSF to partially suppress the proliferation of cells of the human leukaemic cell line U937 and in combination with GM-CSF the proliferation of the human leukaemic cell

lines U937 and HL60;

(d) binds specifically to murine LIF receptors on M1 cells and murine macrophages and competes completely for the binding of murine ^{125}I-LIF to such cells.

(e) binds to specific cellular receptors on the human hepatoma cell line Hep-2G.

Human LIF has also been determined to have the following properties:

(f) is identical to murine LIF at approximately 80% of amino acid residues in the mature protein;

(g) the primary amino acid sequence detailed in Figures 25, 26 and 29 herein;

(h) is encoded by a unique gene bounded in chromosomal DNA by restriction endonuclease cleavage sites as illustrated in Figure 27 herein;

(i) has as a portion of the sequence of its gene, the nucleotide sequence detailed in Figure 25 herein.

In a first aspect of the present invention there is provided a polypeptide having leukaemia inhibitory factor (LIF) activity, characterised in that it is capable of suppressing the proliferation of M1 myeloid leukaemic cells, it comprises the amino acid sequence set forth in Figures 15, 26 or 29 either completely or in part and in that it is capable of competing with a polypeptide of said sequence for binding to specific cellular receptors on M1 cells or murine or human macrophages.

Other aspects of the invention provide:

a polypeptide having leukaemia inhibitory factor (LIF) activity, characterised in that it is capable of suppressing the proliferation of M1 myeloid leukaemic cells, it is encoded by a nucleotide sequence which is capable of hybridising under conditions of 6xSSC, 65°C or conditions of higher stringency, to a sequence of a LIF encoding insert selected from the LIF-encoding inserts of clones pLIF7.2b, pLIFNK1, pLIFNK3, and pHGLIFBamI as defined in Figures 10, 11, 15 or 25 and in that it is capable of competing with a polypeptide of the amino acid sequence set forth in Figures 15, 26 or 29 for binding to specific cellular receptors on M1 cells or murine or human macrophages;

a polypeptide having leukaemia inhibitory factor (LIF) activity characterised in that:

(a) it is capable of suppressing the proliferation of M1 myeloid leukaemic cells;

(b) it comprises the amino acid sequence set forth in Figures 15, 26 or 29 either completely or in part;

(c) it is capable of competing with a polypeptide of said sequence for binding to specific cellular receptors on M1 cells or murine or human macrophages; and

(d) it is encoded by a nucleotide sequence which is capable of hybridising under conditions of 6xSSC, 65°C or conditions of higher stringency, to a sequence of a LIF encoding insert selected from the LIF-encoding inserts of clones pLIF7.2b, pLIFNK1, pLIFNK3, and pHGLIFBamI as defined in Figures 10, 11, 15 or 25; and

a polypeptide having leukaemia inhibitory factor (LIF) activity, characterised in that it is capable of suppressing the proliferation of M1 myeloid leukaemic cells, it comprises the amino acid sequence set forth in Figures 15, 26 or 29 either completely or in part or a sequence which has at least 78% amino acid homology therewith, and in that it is capable of competing with a polypeptide of said sequence for binding to specific cellular receptors on M1 cells or murine or human macrophages.

This invention also provides methods for the production of essentially pure LIF, particularly for the production of essentially pure murine LIF by purification from Krebs II ascites tumour cells, and of essentially pure human LIF by purification from the human bladder carcinoma cell line 5637 (ATCC No. HTB 9).

Thus a further aspect of the invention provides a method of purifying a glycosylated or non-glycosylated LIF polypeptide as defined above, which comprises (a) chromatographing LIF in crude form on an anion exchange column, and eluting LIF by an increasing salt gradient, (b) chromatographing the product of step (a) on a lectin affinity column, said lectin having an affinity for mannose, and eluting LIF with a mannose derivative, (c) chromatographing the product of step (b) on a cation exchange column and eluting LIF with an increasing salt gradient, (d) chromatographing the product of step (c) under HPLC conditions on a reverse phase column and eluting LIF with an increasing gradient of acetonitrile.

Essentially LIF may also be produced by host cells, such as yeast cells, mammalian cells and E.coli, containing recombinant DNA molecules coding for the amino acid sequence of LIF, or part thereof.

The references herein to "in essentially pure form" denote a form of polypeptide or glycopolypeptide in which at least 90% of the polypeptide or glycopolypeptide appear as a single band when electrophoresed on an appropriate sodium dodecyl sulphate polyacrylamide gel, said band being coincident with LIF activity.

In another aspect there is provided a method for isolation of recombinant DNA clones containing nucleotide sequences encoding polypeptides having LIF activity as defined above. The present invention also relates to a nucleotide sequence which has the capacity to encode the unique sequence of amino acids determined to be characteristic of LIF and to furthermore allow the complete amino acid sequence of LIF to be inferred.

In yet another aspect, this invention extends to a recombinant DNA molecule comprising a nucleotide sequence as set forth in Figures 10, 15 (bases 1-630), 25 or 29 (bases 1-543) completely or in part, which encodes a polypeptide capable of suppressing the proliferation of M1 myeloid leukaemic cells and competing with a polypeptide having an amino acid sequence set forth in Figures 15, 26 or 29 for binding to specific cellular receptors on M1 cells or murine or human macrophages. Preferably such DNA molecules contain the aforementioned nucleotide sequences encoding LIF (or substantially similar analogues thereof), either completely or in part, in a form in which said nucleotide sequences are able to direct the synthesis and production of LIF, either completely or in part. This aspect of the invention also extends to cloning vectors (such as plasmids) and host cells having such recombinant DNA molecules inserted therein. Furthermore, the invention enables the preparation of synthetic LIF, either complete or in part, or substantially similar analogues thereof, produced by expression of such recombinant DNA molecules or by peptide synthesis.

DETAILED DESCRIPTION OF THE INVENTION

In the present investigation of LIF, in particular with a view to providing alternative sources of this glycoprotein of both murine and human origin to enable use in clinical and other applications, murine LIF has been purified from Krebs II ascites tumour cells by an efficient purification procedure and has been subjected to partial amino acid sequence analysis as a first step towards chemical or biosynthetic production of this factor.

LIF radioactively derivatized with I^{125} has been used in a receptor competition assay to identify cells and tissues of murine and human origin that synthesize and produce LIF. As a result of this survey, a recombinant DNA library of DNA copies of mRNA from one of these sources has been screened and murine LIF-encoding clones identified by hybridization with radioactively-labelled oligonucleotides encoding portions of the previously determined murine LIF amino acid sequence, and the aforementioned murine LIF cDNA clones subjected to nucleotide sequence analysis. Moreover, the cloned murine LIF encoding sequence has been used as a hybridization probe to identify a human gene encoding a homologue of murine LIF and hybridisation conditions established under which said cross-species hybridization can be effectively performed. As a result, recombinant DNA clones containing DNA sequences encoding the human homologue of murine LIF have been identified.

As a result of this construction of LIF- encoding sequences, recombinant DNA molecules have been constructed that use cloned murine or human LIF- encoding DNA sequences to direct the synthesis of clonally pure LIF, either completely or in part, for example in cultured mammalian cells, in yeast or in bacteria. This invention thus extends to substantially pure LIF produced in this manner.

In one particular embodiment, this invention relates to the expression of the cloned human and murine LIF genes in yeast cells, in fibroblasts and in E.coli, and to the modification of the cloned gene so that it may be so expressed, as well as to the establishment of the biological and biochemical properties of recombinant human and murine LIF.

In this embodiment also, the present invention relates to recombinant DNA molecules containing nucleotide sequences encoding human or murine LIF (or substantially similar analogues thereof), either completely or in part, in a form in which said nucleotide sequences are able to direct the synthesis and production of human or murine LIF in yeast cells, fibroblasts or E.coli, either completely or in part. This invention also provides cloning vectors (such as plasmids) and host cells having such recombinant DNA molecules inserted therein. Furthermore, the invention extends to synthetic human or murine LIF, either complete or in part, or substantially similar homologues thereof, produced by expression of such recombinant DNA molecules in yeast cells. In one embodiment of the present work relating to LIF, the human and murine LIF gene sequences have been modified and installed in a yeast expression vector, YEpsec1. Yeast cells have been transformed with the resulting recombinants and the medium conditioned by yeast cells so transformed has been shown to contain a factor with biological properties analogous to those of native human and murine LIF.

In view of the potential of LIF for use in the treatment of patients with some forms of myeloid leukaemia and patients with certain infections, the present invention also extends to pharmaceutical compositions comprising LIF, particularly human LIF, either completely or in part, produced for example using cloned LIF-encoding DNA sequences or by chemical synthesis, and to pharmaceutical compositions of analogues of LIF, for example produced by chemical synthesis or derived by mutagenesis of aforesaid cloned LIF-encoding DNA sequences. The pharmaceutical compositions may also contain at least one other biological regulator of blood cells, such as G-CSF or GM-CSF. Furthermore, the invention also extends to diagnostic reagents for use in detecting genetic rearrangements, alterations or lesions associated with the human LIF

gene in diseases of blood cell formation, including leukaemia and congenital diseases associated with susceptibility to infection.

EXAMPLE 1

The accompanying Figures 1 to 5 relate to various steps of the purification method described below, showing where LIF is pooled in each fractionation step and showing evidence of its purity. In the Figures:

Figure 1: relates to Step 2 of the purification of LIF on DEAE-Sepharose CL-6B®. The bar indicates fractions that are pooled for Step 3.

Figure 2: relates to Step 3 of the purification of LIF on Lentil lectin Sepharose 4B®. The bar indicates fractions that are pooled for Step 4.

Figure 3: relates to Step 4 of the purification of LIF on CM-Sepharose CL-6B®. The bar indicates fractions that are pooled for Step 5.

Figure 4: relates to Step 5 of the purification of LIF on a fatty acid analysis HPLC column. Fractions from 39.6 to 41.3% acetonitrile are pooled.

Figure 5: shows the molecular weight and purity of purified LIF. The upper panel shows the migration of protein standards (93,000; 67,000; 43,000; 30,000; 20,000 and 14,000 molecular weight) and purified LIF (two preparations) on 8-25% polyacrylamide SDS gel. The protein and biological activity are both associated with a protein of molecular weight 58,000.

Step 1 Krebs II tumour cells (1×6^6) are injected intraperitoneally into C57B16/6fj/ WEHI mice and after 7 days the mice are sacrificed and the peritoneal fluid removed. The cells are centrifuged, resuspended at 5×10^6 cells/ml in Dulbecco's modified Eagle's medium containing E.coli lipopolysaccharide (200ng/ml) and incubated for 24 hrs at 37°C in a humidified incubator containing 10% CO_2 in air. The cells are again centrifuged, the conditioned medium removed, made up to 0.02% (w/v) sodium azide and stored at 4°C. Thirty-six litres of conditioned medium are concentrated to 100ml using a HIP10-8 hollow fibre cartridge in an Amicon DC2A concentrator, exchanged into 10mM Tris-HCl buffer pH8.0 containing 1mM phenylmethyl sulphonyl fluoride (PMSF), sodium azide (0.02% w/v) and Tween 20 (0.02%v/v) and re-concentrated to 20ml over a YM-10 membrane in an Amicon stirred cell.

Step 2. The concentrate is applied to a column (2.5 x 30cm) of DEAE-Sepharose CL-6B (Pharmacia) equilibrated in the same buffer and eluted with 200ml of equilibration buffer followed by a 400ml linear gradient to 0.3M NaCl in the same buffer. The flow rate is 25ml/hr and 5.8ml fractions are collected and assayed. (See Fig.1)

Step 3. The fractions of protein which failed to bind to the gel during the pre-gradient step and which contained activity on murine M1 cells are pooled, concentrated over a YM-10 membrane, exchanged into 100mM sodium acetate buffer pH6.0 containing 1mM $MgCl_2$, 1mM PMSF, 0.2% sodium azide and 0.02% Tween 20 and applied to a column (1 x 4cm) of Lentil lectin-sepharose 4B (Pharmacia) equilibrated in the same buffer. The column is eluted with 25ml of equilibration buffer and then a 100ml linear gradient to 0.5 M α-methyl-D-mannopyranoside in the same buffer at a flow rate of 10ml/hr. Fractions of 3ml are collected and assayed. (See Fig.2)

Step 4. The fractions of proteins which bind to the gel and are eluted by the sugar and have activity on M1 cells are pooled, concentrated and exchanged into 100mM sodium acetate buffer pH5.0- containing 1mM PMSF, 0.02% sodium azide and 0.02% Tween 20 and applied to a column (1.0 x 4.0cm) of CM-Sepharose CL-6B® (Pharmacia) equilibrated in the same buffer. The column is eluted with 25ml equilibration buffer and then a 100ml linear gradient to 1.0m NaCl in the same buffer and then by 25ml of 1.0M NaCl adjusted to pH10 with NaOH. The flow rate is 2.0 ml/hr and 3.0ml fractions are collected. (see Fig.3)

Step 5. The fractions of proteins which bind to the gel and are eluted by the NaCl gradient and have activity on M1 cells are pooled, concentrated to 2ml over a YM-10 membrane and exchanged into 20mM ammonium acetate buffer pH5.0 containing 0.02% Tween 20 and then filtered through a 0.45μ Millipore filter. The pool is loaded into the injector of a high performance liquid chromotography system (Beckman) equipped with a Waters fatty acid analysis column and dual pumps with gradient programmer. The column is equilibrated in water containing 0.1% (v/v) trifluoroacetic acid (TFA), the sample is injected, and the column is then eluted with a 5min linear gradient to 34.8% (v/v) acetonitrile in water and 0.1% TFA and then eluted with a 50min linear gradient to 49.8% (v/v) acetonitrile in water and 0.1% TFA. The flow rate is 1 ml/min and 1ml fractions are collected into polypropylene tubes containing 50μl of 100mM ammonium bicarbonate and 0.4% Tween 20. (see Fig.4)

The fractions with activity on M1 cells from step 5 consisted of two overlapping ultraviolent- absorbing peaks. Each peak was associated with activity on M1 cells and each peak corresponded to a single protein

band of Mr 58,000 on sodium dodecyl sulphate polyacrylamide gels using silver staining. In each case the protein band of Mr 58,000 contained the biological activity on M1 cells as assessed by cutting a parallel track on the gel (also loaded with LIF) into 1mm strips, extracting the protein from the gel strips and assaying the extracted protein for its ability to suppress proliferation and induce differentiation in M1 cells in semi-solid agar cultures. (see Fig.5)

EXAMPLE 2

The following Example sets out the steps used in obtaining the N-terminal amino acid sequence and amino acid sequences of various peptides generated by proteolytic cleavage with trypsin or Staphylococcal V8 protease.

LIF purified by the procedure of Example 1 (15µg) was applied to a microbore (2mm) column packed with Brownlee RP300® beads (C8) and eluted on a HPLC system with a linear gradient from 0-60% acetonitrile in 0.1% trifluoroacetic acid. The protein eluted as a single peak in a volumn of 100µl This was applied to a glass fibre disc and allowed to dry and then placed in the sequencing chamber of an Applied Biosystem (Model 470A) gas phase protein sequencer. The protein was sequenced by Edman chemistry and the phenylthiohydantoin derivatives of the individual amino acids identified by HPLC on an Applied Biosystems 120A PTH analyser.

A separate aliquot of LIF (20µg) also purified by the aforementioned procedure, was diluted to 2ml in 6m guanidine hydrochloride, 0.2M Tris-HCL buffer pH8.5, 2mM dithiothreitol and incubated at 37°C for 2 hrs. Then a 30-fold molar excess of iodoacetic acid was added and the solution incubated at 37°C for a further 15min. The solution was applied to the same microbore HPLC column and eluted as described above. The reduced and carboxymethylated derivative of LIF (RCM-LIF) eluted 3min later from the column than the untreated LIF and RCM-LIF (200µl) was diluted to 1ml with 0.1 M Tris-HCL buffer pH8.0 containing 0.02% Tween 20, 1mM $CaCl_2$ and 2µg of TPCK-treated trypsin. This incubation was allowed to proceed for 18hr at 37°C and the mixture was again applied to the microbore column and eluted in the same way as described above. Individual peptides appeared as UV-absorbing peaks, and were collected individually Some of these peptides were sequenced directly as described above while others were repurified on the same column but using an 0-60% acetonitrile gradient in 0.9% NaCl pH6.0 before being sequenced.

A second aliquot of RCM-LIF (200µl = 10µg) was diluted to 1ml with 0.05M sodium phosphate buffer pH 7.8 containing 0.002M EDTA and 0.02% Tween 20, and was digested with 2µg of Staphylococcus aureus V8 protease for 18 hours at 37°C. The reaction mixture was applied to the same microbore column and eluted as described above.

The 26 amino acids defining LIF from the amino terminal in sequence were found to be:

N-Pro-Leu-Pro-Ile-Thr-Pro-Val-X-Ala-Thr-X-Ala-Ile-
Arg-His-Pro-Cys-His-Gly-Asn-Leu-Met-Asn-Gln-Ile-Lys

The amino acid sequences of several tryptic peptides were found to be:

1. His-Pro-Cys-His-Gly-Asn-Leu-Met-Asn-Gln-Ile-Lys
2. Met-Val-Ala-Tyr ...
3. Gly-Leu-Leu-Ser-Asn-Val-Leu-Cys ..
4. Leu-Gly-Cys-Gln-Leu-Leu-Gly-Thr-Tyr-Lys
5. Val-Leu-Asn-Pro-Thr-Ala-Val-Ser-Leu-Gln-Val-Lys
6. **Asn-Gln-Leu-Ala-Gln-Leu-X-Gly-Ser-Ala-Asn-Ala-Leu-**
   Phe-Leu-Val-Glu-Leu-Tyr ....
7. Leu-Val-Ile-Ser-Tyr...

8.

Val-Gly-His-Val-Asp-Val-Pro-Val-Pro-Asp-His-Ser-Asp-Lys-Glu-Ala-Phe-Gln.

9. Leu-X-Ala-Thr-Ile-Asp-Val-Met-Arg.

10. Gln-Val-Ile-Ser-Val-Val-X-Gln-Ala-Phe.

The amino acid sequence of a peptide generated by digestion of LIF with the V8 protease was:

Ala-Phe-Gln-Arg-Lys-Lys-Leu-Gly-Cys-Gln-Leu-Leu-Gly-Thr-Tyr-Lys-Gln-Val-Ile-Ser-Val-Val-Val-Gln-Ala-Phe.

EXAMPLE 3

The following Example sets out the steps used to obtain radioactively-derivatized LIF and to identify sources of LIF by a receptor competition assay.

The accompanying Figure 6 relates to Example 3: Binding of radioiodinated-pure LIF (approximately 2 x 10^5 cpm/ng) to M1 myeloid leukaemia cells at 0°C. M1 cells (5 x 10^6) were incubated with ^{125}I-LIF (4 x 10^5 cpm) with or without competing factors for 2 hours after which bound radioactivity was separated from unbound radioactivity by centrifugation through foetal calf serum. A. Titration of unlabelled pure LIF and pure Multi-CSF, GM-CSF, G-CSF or M-CSF as competing factors at the indicated dilutions (10µl of each dilution added to a final volume of 80µl).
The highest concentrations of each were 10^4 units/ml; 3.5 x 10^4 units/ml; 3 x 10^4 units/ml; 5 x 10^4 units/ml; 4 x 10^4 units/ml; respectively. B. Ability of pure LIF or lipopolysaccharide (LPS) or concentrated (4-8-fold) media conditioned by various cells or serum from endotoxin-injected mice to compete for the binding of ^{125}I-LIF to M1 cells as above.

LIF, purified from Krebs II ascites cell conditioned medium as described in Example 1, was radioactively labelled with ^{125}I on tyrosine residues as described previously (Nicola, N.A. and Metcalf, D., J.Cell.Physiol. 128:180-188, 1986) producing ^{125}I-LIF with a specific radioactivity of approximately 2 x 10^5 cpm/ng. ^{125}I-LIF bound specifically to M1 myeloid leukaemic cells as well as murine adult bone marrow, spleen, thymus and peritoneal exudate cells. Cell autoradiographic analysis indicated that ^{125}I-LIF bound specifically only to macrophages and their precursor cells, with receptor numbers increasing with cell maturation, and not detectably to any other haemopoietic cell type. This suggests that LIF might have clinically useful applications in modulating macrophage function in a variety of disease states. The specific binding of ^{125}I-LIF to M1 myeloid leukaemic cells or to peritoneal macrophage populations was inhibited by unlabelled LIF, but not by a range of other haemopoietic growth factors, including G-CSF, GM-CSF, M-CSF (CSF-1), Multi-CSF (interleukin 3), interleukins 1,2,4,6, endotoxin or other growth factors (see for example Figure 6). The ability of cell conditioned media or cell extracts to inhibit the binding of ^{125}I-LIF to such cell populations (radioreceptor assay for LIF) (Figure 6B) thus provides a specific assay for the presence of LIF production or storage by various cells and tissues. For example, medium conditioned by lectin-activated LB3 T cells strongly inhibited specific binding of ^{125}I-LIF to M1 cells thus indicating that LB3 cells produce LIF.

EXAMPLE 4

The following Example sets out the steps used to obtain a cloned complementary DNA copy of the mRNA encoding murine LIF.

The accompanying Figures 7 to 10 relate to various steps of the method described below. In the Figures:

Figure 7 relates to Step 1 of the cloning of LIF cDNA clones: the accumulation of mRNAs for haemopoietic growth regulators in the cytoplasm of LB3 cells following stimulation with the lectin concanavalin A. Cytoplasmic polyadenylated RNA (5µg) from WEHI-3B D$^-$ cells (track 1), T cell clone LB3 cells cultured at 10^6 cells/ml with 5µg/ml concanavalin A for 5 hours (track 2), and unstimulated LB3

cells (track 3) was electrophoresed on formaldehyde agarose gels, transferred to nitrocellulose and hybridized with a ^{32}P-GM-CSF probe (panel a) or a ^{32}P-Multi-CSF probe (panel b) (for details see Kelso A., Metcalf, D. and Gough,N.M. J.Immunol. 136:1718-1725, 1986).

Figure 8 relates to Step 3 of the cloning of LIF cDNA clones: Synthetic oligonucleotide used for identifying LIF clones. A portion of the amino acid sequence of murine LIF near the N-terminus (residues 15-26, see Example 2) is shown on the top line, the possible combinations of mRNA sequences that could encode this peptide in the middle, and the oligonucleotide probe complementary to this mRNA sequence, below. Note that for all amino acid residues except Cys 17 and His 18, the oligonucleotide is complementary only to the codons most frequently used in mammalian coding regions (Grantham et al. Nucleic Acids Res. 9:43-77, 1981). For Cys 17, the sequence is complementary to both codons. For His 18, the oligonucleotide is complementary to the less frequent CAU codon. It was considered unlikely that the CAC codon would be used as it would generate, in conjunction with the neighbouring Gly codon, the infrequent CpG dinucleotide (Swartz et.al, J. Biol. Chem. 238:1961-1967, 1962)

Figure 9 relates to Step 4 of the cloning of LIF cDNA clones; the identification of LIF cDNA clones by hybridization with the oligonucleotide illustrated in Figure 8.

Figure 10 relates to Step 5 of the cloning of LIF cDNA clones: nucleotide sequence of cDNA insert pLIF7.2b and LIF amino acid sequence. The sequence of the mRNA-synonymous strand of the cDNA is listed 5′ to 3′ with the predicted amino acid sequence of LIF given above; numbers at the end of lines indicate the position of the final residue (amino acid or nucleotide) on that line. The amino acid sequence is numbered consecutively from the first residue encoded in this clone. Regions of amino acid sequence determined by analysis of the protein are overlined; there are no discordancies between the two. Pro 1 was the N-terminal residue determined by direct amino acid sequence analysis (Example 2). Seven potential N-linked glycosylation sites (Neuberger, A. et al in Glycoproteins, Gottschalk, A. ed. Elsevier, Amsterdam, pp 450-490, 1972) are indicated with asterisks and four potential O-linked glycosylation sites (Takahashi, N. et al, Proc. Natl. Acad. Sci. USA, 81: 2021-2025, 1984) with hatches.

Step 1: Preparation of RNA containing the LIF mRNA.

Cells of the cloned murine T cell line LB3 (Kelso, A. and Metcalf, D. Exptl.Hematol. 13:7-15. 1985) were stimulated with the lectin concanavalin A for 6 hours to enhance the accumulation in the cytoplasm of mRNAs encoding various factors which regulate the growth and differentiation of haemopoietic cells (Kelso A., Metcalf, D. and Gough, N.M. J.Immunol. 136:1718-1725, 1986). (For example, Figure 7 shows the increased production of mRNAs encoding the haemopoietic growth factors GM-CSF and Multi-CSF in cells so stimulated). Cytoplasmic RNA was prepared from 5 x 10^8 concanavalin A-stimulated LB3 cells using a technique described previously (Gough, N.M. J.Mol.Biol. 165:683-699. 1983). Polyadenylated mRNA molecules were partitioned from ribosomal RNA by two cycles of chromatography on oligo-dT cellulose using standard procedures (Maniatis, T. et.al., Molecular Cloning, Cold Spring Harbor, New York, 1982)

It was found subsequently by Northern blot analysis that unlike the mRNAs for GM-CSF and Multi-CSF the LIF mRNA is present constitutively in LB3 cells and its abundance is not enhanced by concanavalin A stimulation (Gearing D.P. et al. EMBO J. 6: 3995-4002, 1987).

Step 2: Synthesis and cloning of a library of LB3 cDNA molecules.

Double-stranded DNA copies of the LB3 mRNA prepared as described above were synthesized by standard procedures (Maniatis, T. et.al., Molecular Cloning, Cold Spring Harbor, New York 1982 and Gough, N.M. et.al. Nature 309:763-767, 1984). Briefly, 10µg of cytoplasmic polyadenylated LB3 mRNA were used as a template for synthesis of single-stranded complementary DNA (cDNA) in a reaction catalyzed by avian myeloblastosis virus reverse transcriptase and primed with oligo-dT. After completion of this reaction, the mRNA was degraded by incubation at 65°C for 1 hour in 0.3 M NaOH, 1mM EDTA. After neutralisation of the base and recovery of the cDNA by ethanol precipitation, the single-stranded cDNA was converted to duplex form in a reaction catalyzed by the Klenow fragment of E.coli DNA polymerase I. The "hairpin loop" structure of the cDNA was then cleaved by treatment with the single-strand specific nuclease S1 and then tails of deoxycytidine residues (approximately 20-30 residues long) were appended to each end of the double-stranded cDNA using the enzyme terminal deoxynucleotidyltransferase as described (Michelson, A.M. and Orkin, S. J. Biol.Chem. 257:14773-14782, 1982). The dC-tailed cDNA was fractionated by electrophoresis on a 1.5% agarose gel and molecules greater than 500bp in length recovered and annealed to a plasmid DNA molecule (pJL3, Gough, N.M. et.al., EMBO J. 4:645-653. 1984) that had been cleaved with the restriction endonuclease Sac 1 and to which tails of deoxyguanosine residues had been

appended (Michelson, A.M. and Orkin, J., J.Biol.Chem. 257:14773-14782, 1982). The tailed cDNA and plasmid molecules were annealed as described (Gough, N.M. et.al., Biochemistry 19:2702-2710, 1980) and E.coli MC1061 (Casadaban, M. and Cohen, S., J.Mol.Biol. 138:179-207, 1980) transformed with the annealed cDNA/plasmid mixture and approximately 50,000 independently transformed bacterial colonies selected by growth on agar plates containing 10µg/ml ampicillin. The transformed bacterial colonies were removed from the agar plates by washing with liquid growth medium containing 50µg/ml ampicillin and stored as 10 independent pools in 10% glycerol at -70°.

Step 3: Design of oligonucleotide probes.

A sequence of 26 amino acids at the amino terminus of LIF and residues from 11 different peptides derived by digestion of LIF with trypsin and V8 protease have been determined (see Example 2). These amino acid sequences provided the basis for the design of oligonucleotides complementary to certain defined regions of the mRNA encoding LIF for use as hybridization probes to identify cDNA clones corresponding to the LIF mRNA.

Each naturally occurring amino acid is encoded in its corresponding mRNA by a specific combination of 3 ribonucleoside triphosphates (a codon) (eg Watson, J. Molecular Biology of the Gene, 3rd ed., Benjamin/Cummings, Menlo Park, Calif., 1976). Certain amino acids are specified by only one codon, whereas others are specified by as many as 6 different codons (eg Watson, J. Molecular biology of the Gene, supra). Since therefore a large number of different combinations of nucleotide sequences could in fact encode any particular amino acid sequence, a large number of different degenerate oligonucleotides would need to be constructed in order to cater for every possible sequence potentially encoding that peptide. There are however, certain technical constraints in the use of highly degenerate oligonucleotides as hybridization probes. However, since for a given amino acid not all codons are employed with equivalent frequency (Grantham, R. et.al. Nucleic Acids Res. 9:43-73, 1981) and since in the mammalian genome the CpG dinucleotide is underrepresented, occurring at only 20-25% of the frequency expected from base composition (Swartz, M.N. et.al., J.Biol.Chem. 238:1961-1967, 1962), it is often possible to predict the likely nucleotide sequence encoding a given peptide and thus reduce the complexity of a given oligonucleotide probe. Given the foregoing considerations, a number of oligonucleotides corresponding to different LIF peptides were designed and synthesized by standard procedures.

Step 4: Screening of an LB3 cDNA library for LIF-encoding clones.

For screening colonies of bacteria by hybridization with oligonucleotide probes, 10,000-15,000 bacterial colonies from each pool of the aforementioned LB3 cDNA library were grown on agar plates (containing 50µg/ml ampicilin), transferred to nitrocellulose filter disks and plasmid DNA amplified by incubation of the filters on agar plates containing 200µg/ml chloramphenicol (Hanahan, D. and Meselson, M. Gene 10:63-67, 1980). After regrowth of colonies on the original plate, a second nitrocellulose filter was prepared as above. The master plate was regrown a second time and then stored at 4°C. Plasmid DNA was released from the bacterial colonies and fixed to the nitrocellulose filters as previously described (Maniatis, T. et.al. Molecular Cloning, Cold Spring Harbor, New York, 1982). Before hybridization, filters were incubated for several hours at 37°C in 0.9M NaCl, 0.09 M sodium citrate, 0.2% Ficoll., 0.2% polyvinyl-pyrollidone, 0.2% bovine serum albumin, 50µg/ml heat denatured salmon sperm DNA, 50µg/ml E.coli tRNA, 0.1M ATP and 2mM sodium pyrophosphate. Hybridization was performed in the same solution, containing in addition 0.1% NP40, at 37°C for 18 hours. 500ng of the synthetic oligonucleotide shown in Figure 8, was radioactively labelled in a reaction catalyzed by polynucleotide kinase and containing 500 µCi of [γ-^{32}P]ATP (specific activity 2,000-3,000 Ci/mmol). Unincorporated [γ-^{32}P]ATP was then separated from the radioactively labelled oligonucleotide by ion exchange chromatography on a NACS-PREPAC column (Bethesda Research Laboratories) according to the manufacturer's instructions. The radioactively labelled oligonucleotide was included in the hybridization reaction at a concentration of approx.20ng/ml. After hybridization, filters were washed extensively in 0.9M NaCl, 0.09M sodium citrate, 0.1% sodium dodecyl sulphate at various temperatures (as described below) and after each wash, autoradiographed.

The rationale behind performing successive rounds of washing was that at lower temperatures, all clones having even a small degree of homology with the oligonucleotide (as little as approximately 15 nucleotides) would be revealed as autoradiographic spots appearing on duplicate filters. As the temperature is increased, the oligonucleotide probe would be melted from clones with the lowest level of homology and the corresponding autoradiographic signals would thus be lost. Clones with the highest degree of homology (and thus clones which represent the best candidates for containing LIF cDNA sequences) will retain

hybridization at the highest temperature. By this strategy it is thus possible to focus directly on the strongest candidate clones. Figure 9 shows the performance of a set of clones on a duplicate pair of filters containing 15,000 LB3 cDNA clones as the washing temperature was raised from 46 °C to 66 °C. Several clones retained hybridization to the oligonucleotide only at lower temperatures whereas several retained hybridization even at 66 °C (for example, clones 1 and 2). The latter clones were thus selected for further analysis and the corresponding bacterial colonies removed from the master plates, purified and plasmid DNA prepared from each bacterial clone by standard procedures (Maniatis, T. et.al. Molecular Cloning. Cold Spring Harbor, 1982). Preliminary structural analysis of these clones (by assessing the size of the inserted cDNA, by mapping the locations of cleavage sites for various restriction endonucleases and by testing for hybridization with various oligonucleotides corresponding to different LIF peptides) indicated that each of these clones were in fact identical; that is, they represented different isolates of the same original cloning event. Thus further detailed analysis was performed on only one clone, pLIF7.2b.

Step 5: Determination of the nucleotide sequence of pLIF7.2b.

Nucleotide sequence analysis of the cDNA portion of clone pLIF7.2b was performed by the dideoxy chain termination method (Sanger, F. et.al., Proc.Natl.Acad.Sci. USA 74:5463-5467, 1977), using alkaline or heat denatured double-stranded plasmid DNA as template, a variety of oligonucleotides complementary both to the regions of the vector (pJL3) flanking the cDNA and to sequences within the cDNA insert as primers, and using both the Klenow fragment of E.coli DNA polymerase I and the avian myeloblastosis virus reverse transcriptase as polymerases in the sequencing reactions. The sequence so determined for the cDNA portion of clone pLIF7.2b is shown in Figure 10. Such analysis confirmed that this clone does indeed contain a DNA copy of a mRNA with the capacity to encode the LIF molecule, since within the only translational reading frame that spans the entire cDNA uninterrupted by stop codons, all of the amino acid sequences previously determined for various peptides of the LIF molecule may be found. This clone does not however contain a complete copy of the LIF coding region since (a) it does not extend at the 5′ end through a region encoding a presumed hydrophobic leader sequence initiated by a methionine codon and (b) it does not include at its 3′ end an in-frame translational stop codon. It does however extend at the 5′ end past the start of the region encoding the mature protein, determined by comparison with the previously determined amino-terminal amino acid sequence (residue Pro 9 in Figure 10).

EXAMPLE 5

The following sets out the steps used to construct a full length copy of the murine LIF coding region, to install this coding region in a yeast expression vector and to produce murine LIF.

The accompanying Figures relate to various steps of the method described below. In the Figures:

Figure 11: relates to Step 1 of the expression of murine LIF in yeast cells: C-terminal amino acid sequence of LIF. The amino acid sequence at the C-terminus of the pLIF7.2b reading frame is shown above the sequence of a Staphylococcal V8 protease and a tryptic peptide derived from LIF purified from Krebs ascites tumour cells. Note that these latter peptides extend the pLIF7.2b LIF sequence by 9 amino acids and terminate at the same residue. The nucleotide and amino acid sequence at the corresponding region of clone pLIFNK1 is shown at the bottom line, confirming the C-terminus assigned by direct amino acid sequencing. The numbering system is that of Figure 10.

Figure 12: relates to step 2 of the expression of murine LIF in yeast cells: reconstruction of the pLIF7.2b cDNA for insertion into the yeast expression vector, YEpsec1. Partial nucleotide sequence of the pLIF7.2b cDNA and its encoded amino acid sequence are shown on the top line. Numbering is according to Figures 10 and 11. The second and third lines show the sequence of pLIFmut1 and pLIFmut2 respectively. Asterisks indicate stop codons. Restriction sites (Bam HI, Hind III and Eco R1) used for cloning into YEpsec1 (Baldari. C. et al, EMBO J. 6: 229-234, 1987) and pGEX-2T (Smith, D.B and Johnson, K.G., Gene, in press, 1988) are indicated. The bottom line shows partial sequence of the K.lactis killer toxin signal sequence in YEpsec1. The sequence Gly-Ser encoded at the Bam HI restriction site is efficiently recognized by signal peptidase (Baldari, C. et al, EMBO J. 6: 229-234, 1987).

Figure 13: relates to step 4 of the expression of murine LIF in yeast cells: the biological activity of yeast-derived recombinant LIF in cultures of M1 leukaemic cells. Titration in M1 cultures of yeast-derived recombinant LIF (-●-) versus purified native LIF-A. (-o-) showing similar concentration dependent induction of differentiation in M1 colonies (panel A) and suppression of colony formation (panel B). Each point represents the mean value from duplicate cultures.

Figure 14: relates to step 5 of the expression of murine LIF in yeast cells: the ability of different dilutions of authentic native murine LIF-A (- o -), conditioned medium from yeast cells containing the LIF-mut1/YEpsec1 recombinant induced with galactose (- ● -) and conditioned medium from the same yeast cells, but not induced with galactose (- + -) to compete for the binding of native ^{125}I-LIF-A to cellular receptors on murine peritoneal cells.

Step 1: Determination of the amino acid sequence at the C-terminus of murine LIF.

The cDNA clone pLIF7.2b of Example 4 contains an incomplete copy of the murine LIF mRNA At the 5′ end, the cDNA encodes 8 residues N-terminal to the first residue determined by N-terminal amino acid sequence analysis (Pro 9 in Figure 10). At the 3′ end however, pLIF7.2b is incomplete as it does not contain an in-frame translational stop codon. Inspection of the amino acid sequence of two LIF peptides determined by direct amino acid sequencing (Example 2) suggested that pLIF7.2b was lacking only 27 nucleotides of coding region at the 3′ end. As illustrated in Figure 11, a V8-derived peptide started at residue Ala 162 of the cDNA sequence and extended the amino acid sequence deduced from the cDNA clone by 9 residues. A tryptic peptide contained within the V8 peptide terminated at the same residue, suggesting that Phe 187 is the C-terminal residue of the protein. In order to confirm this conclusion, a LIF cDNA clone overlapping with pLIF7.2b and extending 3′-ward was isolated and subjected to nucleotide sequence analysis.

In order to construct and screen an appropriate cDNA library from which to isolate such a clone, a series of different mRNA samples were screened by Northern blot hybridization to identify the RNA samples with the highest concentration of LIF mRNA molecules. Cytoplasmic polyadenylated RNA, prepared essentially as described previously (Gough, N.M. J.Mol Biol. 165: 683-699, 1983), was fractionated on 1% agarose gels containing 20mM morpholinopropane sulfonic acid (MOPS), 5mM sodium acetate, 1mm EDTA (pH 7.0), plus 6% v/v formaldehyde, filters containing RNA were soaked in 2 x SSC, containing 0.2% Ficoll, 0.2% polyvinyl- pyrollidone, 0.2% bovine serum albumin, 2mM sodium pyrophosphate, 1mm ATP, 50μg/ml denatured salmon sperm DNA and 50μg/ml E.coli tRNA, at 67°C for several hours. Hybridization was in the same buffer plus 0.1% SDS at 67°C. The probe used to detect LIF transcripts consisted of an approx.750 bp Eco RI - Hind III fragment containing the cDNA insert of pLIF7.2b subcloned in pSP65. This fragment contains not only the cDNA sequence but also G-C tails and approx.150 bp of pJL3 vector sequence. Riboprobes of approx.2 x 10^9 cpm/μg were derived by transcription of this SP6 subclone using reagents supplied by BRESA (Adelaide). The probe was included in hybridization at approx.2 x 10^7 cpm/ml. Filters were washed extensively in 2 x SSC, 2mM EDTA, 0.1% SDS at 67°C and finally in 0.2 x SSC at 67°C prior to autoradiography.

Such analysis revealed that LIF transcripts were present at low levels in a wide variety of haemopoietic cell lines and that there was considerable variation in the level of LIF mRNA in different batches of Krebs RNA. Two batches of Krebs ascites tumour cell RNA were selected for synthesis of two cDNA libraries. cDNA libraries were constructed using the reagents supplied by Amersham (Product numbers RPN.1256 and RPN.1257) and using the manufacturer's instructions; the cloning vector was λGT10. Approximately 4 x 10^5 recombinant clones were obtained and screened by hybridization with an oligonucleotide corresponding to a sequence of 36 residues at the 3′ end of the pLIF7.2b sequence: nucleotides 500-535 (inclusive) in Figure 10.

Phage plaques representing the Krebs cDNA library were grown at a density of approx.50,000 plaques per 10 cm petri dish, transferred in duplicate to nitrocellulose and treated using standard techniques (Maniatis, T. et al, Molecular Cloning. Cold Spring Harbor, 1982). Prior to hybridization, filters were incubated for several hours at 37°C in 6 x SSC (SSC = 0.15M NaCl, 0.015M sodium citrate), 0.2% Ficoll, 0.2% polyvinyl-pyrollidone, 0.2% bovine serum albumin, 2mM sodium pyrophosphate, 1mM ATP, 50 mg/ml denatured salmon sperm DNA and 50 μ/ml E.coli tRNA. Hybridization was in the same solution containing 0.1% NP40, at 37°C for 16-18 hours. The aforementioned oligonucleotide probe, radioactively labelled using [γ-^{32}P] ATP and polynucleotide kinase to a specific activity of approx.10^9 cpm/μg and separated from unincorporated label by ion exchange chromatography on a NACS column (Bethesda Research Laboratories), was included in the hybridization at a concentration of approx.20 ng/ml. After hybridization, filters were extensively washed in 6 x SSC, 0.1% sodium dodecyl sulphate at 60°C. One plaque representing clone λLIFNK1, positive on duplicate filters, was picked and rescreened at lower density, as before.

The cDNA insert of approx.950 bp in λLIFNK1, which hybridized with the aforementioned oligonucleotide, was subcloned into a plasmid vector (pEMBL8+, Dente, L. et al, Nucl.Acids Res. 11: 1645-1655, 1983) to generate clone pLIFNK1. Nucleotide sequence analysis of the cDNA insert in pLIFNK1 was performed by the dideoxy chain termination method (Sanger, F. et al, Proc Natl.Acad.Sci. USA 74: 5463-

5467, 1977) using alkaline denatured double-stranded plasmid DNA as template (Chen, E Y. and Seeburg P.H., DNA 4: 165-170, 1985), a variety of oligonucleotides complementary both to regions of the vector flanking the cDNA and to sequences within the cDNA insert as primers, and using both the Klenow fragment of E.coli DNA polymerase and AMV reverse transcriptase as polymerases in the sequencing reactions. The nucleotide sequence of a portion of the cDNA insert in pLIFNK1 is shown in Figure 11 The amino acid sequence specified by pLIFNK1 is identical at the C-terminus with the 9 amino acids predicted by direct amino acid sequencing to constitute the C-terminus of LIF, and confirms that Phe 187 is the C-terminal residue of LIF, since in the pLIFNK1 cDNA sequence the codon for this residue is immediately followed by an in-frame translational stop codon.

Step 2: Construction of a LIF codon region in a yeast expression vector.

Initial production of the protein encoded by the LIF cDNA clones was achieved in a eukaryotic system (yeast), so that the expressed product would be glycosylated, secreted and correctly folded The expression vector used, YEpsec1 (Baldari, C. et al, EMBO J. 6: 229-234, 1987), provides an N-terminal leader sequence derived from the killer toxin gene of Kluyveromyces lactis, shown previously to direct the efficient secretion of interleukin 1 (Baldari, C. et al, EMBO J. 6: 229-234, 1987), transcribed from a galactose-inducible hybrid GAL-CYC promoter.

In order to express the protein encoded by pLIF7.2b in this vector, it was necessary to modify the cDNA in several ways (see Figure 12). At the 5′ end it was necessary not only to remove the few nucleotides specifying the partial mammalian leader sequence, but also to include an appropriate restriction endonuclease cleavage site (Bam HI) to allow insertion in-frame with the K.lactis leader and retain an appropriate signal peptidase cleavage site (Gly-Ser). At the 3′ end, two versions of the coding region for LIF were constructed. One version (LIFmut1) was constructed so that a stop codon immediately followed the last codon of pLIF7.2b (Gln 178). The other version (LIFmut2) was constructed to append a sequence encoding the 9 amino acid residues known to be missing from pLIF7.2b (see above), followed by an in-frame translational stop codon. An appropriate restriction endonuclease cleavage site (Hind III) completes both constructs. All of these modifications were achieved by oligonucleotide- mediated mutagenesis The approx.750 bp Eco RI - Hind III fragment carrying the 535 bp cDNA insert of pLIF7.2b, bounded by G-C tails and a portion of the pJL3 vector was subcloned into plasmid pEMBL8+ (Dente, L. et al, Nucl Acids Res. 11: 1645-1655, 1983) and single-stranded DNA was prepared as described (Cesarini, G and Murray J.A.H. in Setlow, J.K. and Hollaender, A. (eds), Genetic Engineering: Principles and Methods. Plenum Press New York, Vol. 8, 1987 (in press)). In vitro mutagenesis was performed as described (Nisbet, I T and Beilharz, M.W. Gene Anal.Techn. 2: 23-29, 1985), using oligonucleotides of 35, 51 and 61 bases respectively, to modify the 5′ end and the 3 end of the cDNA as outline above. Bam HI - Hind III fragments containing the modified LIF cDNA sequences were ligated into plasmid YEpsec1 and the nucleotide sequence of the inserts in the resulting recombinants was determined.

Step 3: Introduction of the YEpSec1/LIF recombinants into yeast cells.

S.cerevisiae strain GY41 (leu2 ura3 ade1 his4 met2 trp5 gal1 cir+; x 4003-5b from Yeast Genetic Stock Centre, Berkeley) was transformed by the polyethylene glycol method (Klebe, R.J. et al, Gene 25 333-341 1983). Transformants were selected and maintained on synthetic minimal medium (2% carbon source 0.67% yeast nitrogen base (Difco) supplemented with 50µg/ml of the required amino acids) under uracil deprivation. Expression of insert sequences in plasmid YEpsec1 was achieved by growing transformants in either non-selective complete medium (1% yeast extract, 2% peptone) or in synthetic minimal medium each containing 2% galactose.

Step 4: Determination of the biological properties of yeast-derived LIF.

Assays for differentiation-inducing activity and leukaemia-suppressive activity of yeast conditioned medium were performed in 1 ml cultures containing 300 M1 cells (provided by Dr. M. Hozumi, Saitama Cancer Research Centre, Japan) in Dulbecco's Modified Eagle's Medium with a final concentration of 20% foetal calf serum and 0.3% agar. Material to be assayed was added in serially diluted 0.1 ml volumes to the culture dish prior to the addition of the cell suspension in agar medium. Cultures were incubated for 7 days in a fully humidified atmosphere at 10% CO_2. Cultures were scored using a dissection microscope at ×35 magnification, scoring as differentiated any colonies with a corona of dispersed cells or composed wholly of dispersed cells. Morphological examination of colonies was performed by fixing the entire culture with 1 ml

2.5% glutaraldehyde then staining the dried cultures on microscope slides using acetylcholinesterase/Luxol Fast Blue/Haematoxylin.

Assays for colony stimulating activity were performed using 75,000 C57BL bone marrow cells as described previously (Metcalf, D. The Hemopoietic Colony Stimulating Factors Elsevier, Amsterdam, 1984). Assays for differentiation inducing activity on WEHI-3B D^+ cells were performed as described previously (Nicola, N. et al, J.Biol.Chem. 258: 9017-9023, 1983).

Medium from cultures of yeast containing the full-length coding region (LIFmut2), but not from cultures of non-transformed yeast, yeast containing the vector YEpsec1 alone, or yeast containing the incomplete coding region (LIFmut1), was able to induce typical macrophage differentiation in cultures of M1 colonies (Figure 13A). As with purified native LIF, with increasing concentrations the yeast-derived material also progressively reduced the number and size of M1 colonies developing (Figure 13B). Comparison with purified native LIF indicated that the yeast conditioned medium contained up to 16,000 Units/ml (approximately 130ng/ml) of LIF. Yeast-derived LIF, like purified native LIF-A, failed to stimulate colony formation by normal granulocyte-macrophage progenitor cells or to induce differentiation in, or suppress proliferation, of WEHI-3B D^+ leukaemic colonies.

Size fractionation on a Sephacryl S-200 column, indicated that yeast-derived LIF co-eluted with proteins of apparent molecular weight 67,000 to 150,000 Daltons, compared with 58,000 Daltons for LIF derived from Krebs cells.

The absence of LIF activity in medium conditioned by yeast containing an incomplete coding region (LIFmut1) suggests that the nine hydrophobic C-terminal residues missing from this construct might be required for LIF function. Whilst these residues might interact with the receptor, they may also constitute part of a hydrophobic core within the protein, and thus their absence may prevent proper folding. Alternatively they might in some way be required for efficient secretion of LIF.

Step 5: Receptor binding specificity of yeast- derived murine LIF and purified native LIF-A from Krebs II ascites cell conditioned medium.

Purified murine LIF-A (Example 1) was iodinated as disclosed previously (Example 3). Peritoneal cells were harvested by lavage from mice in which a high level of macrophages had been induced in the peritoneal cavity with thioglycollate. These cells were washed and resuspended at 2.5×10^6/50µl in Hepes-buffered RPMI medium containing 10% foetal calf serum> Cells in 50µl aliquots were incubated with 200,000 cpm of ^{125}I-LIF-A (10µl, in the same medium) and 10µl of control medium or serial two-fold dilutions of unlabelled pure murine LIF-A or yeast-derived murine LIF. At appropriate concentrations, supernatant from galactose-induced yeast containing the LIFmut2 construct (Figure 12) competed for binding of ^{125}I-LIF-A to its receptors on peritoneal cells while uninduced yeast supernatant did not (Figure 14). The degree of competition was equal to that of authentic native LIF-A, indicating that the yeast-derived LIF-A contained all the information required for binding to LIF-A cellular receptors.

EXAMPLE 6

The following example sets out the steps used to express recombinant murine LIF in mammalian cells.

The accompanying Figure 15 relates to step 1 of the method described below: The nucleotide sequence of the cDNA portion of clone pLIFNK3. The nucleotide sequence of the mRNA-synonymous strand is presented in the 5′ to 3′ orientation with the inferred amino acid sequence of LIF given above. The N-terminal amino acid residue previously determined is indicated as +1. The Eco R1 site at the 5′ end of the cDNA and the Xba I site spanning the stop codon (used in inserting the LIF coding region into pMP-Zen in step 2) are indicated.

Step 1: Determination of the amino acid sequence of the N-terminal leader sequence of murine LIF.

The cDNA clones pLIF7.2b and pLIFNK1 (see above) contain incomplete copies of the LIF mRNA. Although they contain, together, the complete coding region for the mature portion of the LIF protein, they do not contain the complete hydrophobic leader sequence, required for secretion of LIF from mammalian cells.

In order to isolate a cDNA clone containing the region encoding the hydrophobic leader, a further 10^6 cDNA clones constructed as above (Example 5), using the same batch of Krebs mRNA as a template, were screened (as above) using as probes two oligonucleotides corresponding to a sequence of 35 residues at the 5′ end and 36 residues at the 3′ end of the pLIF7.2b sequence (nucleotides 67-102 and 500-535,

inclusive, in Figure 10).

Two plaques representing clones λLIFNK2 and λLIFNK3, positive on duplicate filters were picked and rescreened at lower density, as previously. Since λLIFNK3 was shown to hybridize with each of the two oligonucleotides used, it was selected for further analysis.

The cDNA insert of approx.1400bp in λLIFNK3 was subcloned into the plasmid vector pEMBL 8⁺ (Dente, L., et.al., Nucleic Acids Res. 11:1645-1655, 1983) to generate clone pLIFNK3. Nucleotide sequence analysis of the cDNA insert of pLIFNK3 was performed as for pLIF7.2b and pLIFNK1 (above).

The nucleotide sequence of the cDNA insert in pLIFNK3 is shown in Figure 15, and indicates that pLIFNK3 contains a complete LIF coding region: there is an initiation codon (AUG) at position 23-25 in the pLIFNK3 sequence, preceding a sequence encoding a typical hydrophobic leader sequence of 24 amino acid residues. The coding region extends to the same translational stop codon as defined previously by pLIFNK1 (above).

Step 2: Introduction of a LIF coding region into a mammalian expression vector.

The mammalian expression vector chosen initially was the retroviral expression vector pMP-Zen. The vector is derived from the Moloney murine leukaemia virus-based vector pZIPNeo SV(X) (Cepko, C.L. et.al., Cell 37:1053-1062, 1984) by deletion of the neo^R gene together with nearby SV40 and plasmid sequences, leaving an Xho I expression site. The 3′ region of the vector is also modified to incorporate the enhancer from the long terminal repeat (LTR) of the myeloproliferative sarcoma virus (MPSV) (Bowtel, D.D.L. et.al., Mol.Biol.Med. 4:229-250, 1987). This vector was chosen, firstly since the LIF/PMP-Zen recombinant may be packaged into helper-free infectious retroviral particles by passage through ψ2 cells (Mann, R., et.al. Cell. 33:153-159, 1983). Such viral particles may then be used to efficiently introduce (by infection) the LIF/pMP-Zen recombinant into a wide variety of murine cell types (Mann, R. et.al. Cell 33:153-159, 1983). Secondly, this particular vector, which employs the MPSV LTRs for expression of the foreign coding region, has been shown to direct the efficient expression of certain other haemopoietic growth and differentiation factors including GM-CSF.

The segment of pLIFNK3 chosen for insertion into pMP-Zen extended from position 1 (an Eco R1 site) to position 630 (an Xba 1 site spanning the stop codon) - see Figure 15. This segment was chosen since it contains little more than the coding region of pre-LIF and excludes all of the 3′ untranslated region, which may contain sequences conferring mRNA instability (e.g. Shaw, G. and Kamen, R. Cell 46:659-667, 1986; Verma, I.M. and Sassone-Corsi, P., Cell 51 : 513-514, 1987). In order to insert this segment into pMP-Zen, it was first inserted between the Eco R1 and Xba I sites of pIC20H (Marsh, J.L. et.al. Gene 32: 481-485, 1984) by standard techniques (Maniatis et.al., 1982, supra). The cDNA insert was then recovered from the polylinker of the pIC20H plasmid by Sal I plus Xho I digestion, thus generating a LIF cDNA fragment with cohesive ends appropriate for inserting into the Xho I cloning site of pMP-Zen. The insertion of the LIF cDNA fragment into pMP-Zen was achieved by standard techniques (Maniatis et.al., 1982, supra).

Step 3: Introduction of the pMP-Zen/LIF recombinant into murine fibroblasts.

pMP-Zen/LIF DNA was introduced into ψ2 fibroblasts by electroporation (Potter et.al., PNAS 81:7161-7165, 1984). 30μg pMP-Zen/LIF DNA plus 3μg pSV2Neo DNA (Southern, P.J. and Berg, P. J.Mol.App.Genet. 1:327-341, 1982) were mixed with 1 × 10^6 ψ2 fibroblasts in 1ml of DME/10% FCS (Dulbeccos modified Eagles medium containing 10% foetal calf serum) and subjected to a pulse of 500v at a capacitance of 25μF (using a BioRad Gene-Pulser model No. 1652078). Transfectants were initially selected on the basis of resistance to the antibiotic G418 conferred by the pSV2Neo DNA. G418-resistant ψ2 cells were selected in 400μg/ml G418 by standard procedures (Mann, R. et al, Cell 33: 153-159, 1983). Of 19 G418-resistant clones examined, 2 also contained the pMP-Zen/LIF construct as assessed by LIF activity detectable in ψ2 conditioned media.

Step 4: Determination of the biological properties of ψ2-derived LIF.

Assays for differentiation-inducing activity and leukaemia suppressive activity of conditioned medium from pMP-Zen/LIF containing ψ2 cells were performed as described previously. Medium from cultures of 10^6 ψ2 cells in 3ml DME/10% FCS were assayed for differentiation-inducing activity. The results for two positive cultures are presented in the following table:

Clone No.	Ml Differentiation-inducing Activity (Units/10^6 cells/ml C.M.)
ψ2-control	not detectable
ψ2-11a	>16,000
ψ2-11d	>16,000

Thus, significant levels of biologically active, recombinant murine LIF can be produced in this expression system.

Step 5: Transmission of pMP-Zen/LIF retrovirus from ψ2 cells to haemopoietic cells.

Infectious pMP-Zen/LIF retrovirus could be transferred from the parent ψ2 cell lines to haemopoietic cells of the line FDC-P1 (Dexter, T.M. et.al., J.Exp.Med. 152:1036-1047, 1980) by cocultivation. 10^6 ψ2 cells were mixed with 10^6 FDC-P1 cells in 10ml DME/10% FCS containing the optimal concentration of WEHI-BD$^-$ conditioned medium for growth of the FDC-P1 cells. Following 2 days incubation, the non-adherant FDC-P1 cells were removed, washed free from all adherant ψ2 cells and grown for 16 hours in 3ml of the same medium. Conditioned medium was harvested and assayed for differentiation-inducing and leukaemia suppressive activity as described previously. The results are shown in the following table:

FD Culture derived from Clone No.	Ml Differentiation-Inducing Activity (units/10^6 FDC-P1 cells)
ψ2-control	not detectable
ψ2-11a	approx. 3,000
ψ2-11d	approx. 1,500

Thus the ψ2 clones, ψ2-11a and ψ2-11d, are capable of transmitting biologically active pMP-Zen/LIF retrovirus to haemopoietic cells. These clones should therefore be applicable to the infection of normal murine haemopoietic progenitor cells which can be used to reconstitute the haemopoietic system of irradiated mice in order to study the effects of high level expression of native LIF on normal murine haemopoiesis, in a manner analogous to that used for Zen/GM-CSF viral infection.

EXAMPLE 7.

The following sets out the steps used to express recombinant murine LIF in E.coli cells.

The accompanying Figure 16 relates to step 1 of the method described below: The nucleotide sequence at the glutathione S-transferase/thrombin cleavage site/LIF junction in plasmid pGEX-2T/LIF, and the sequence at the junction of the encoded fusion protein. The C-terminus of the glutathione S-transferase, the thrombin cleavage site and the N-terminus of the murine LIF portions of the tripartite fusion protein, along with the nucleotide sequence encoding this amino acid sequence, are shown. The expected site of cleavage by thrombin is indicated by an arrow.

Step 1: Introduction of a LIF coding region into an E.coli expression vector.

The expression vector used for expressing LIF in E.coli was pGEX-2T (Smith, D.B. and Johnson, K.S., Gene 67, 31-40, 1988), which directs the synthesis of foreign polypeptides as fusions with the C-terminus of Sj26, a 26kD glutathione S-transferase (E.C. 2.5.1.18) encoded by the parasitic helminth Schistosoma japonicum. In the majority of cases, fusion proteins have been shown to be soluble in aqueous solutions and can be purified from crude bacterial lysates under non-denaturing conditions by affinity chromatography on immobilized glutathione. The particular vector pGEX-2T has been engineered so that the glutathione S-transferase carrier can be cleaved from the fusion protein by digestion with the site-specific

protease thrombin.

The complete coding region of murine LIF, derived from plasmid pLIFmut2 (see Example 5 and Figure 12 above) was introduced as a Bam HI - EcoRI fragment into the multiple cloning site of pGEX-2T (Smith D.B. and Johnson, K.S., supra), thus positioning the LIF coding region 3′ of, and in the same translational reading frame as, that of the glutathione S-transferase and the thrombin cleavage site. Thus the LIF protein would be located C-terminal to these elements in a tripartite glutathione S-transferase/thrombin cleavage site/LIF fusion protein (see Figure 16). Note that the position of the thrombin cleavage site is such that two amino acid residues (Gly-Ser) will be appended to the N-terminus of the LIF protein after thrombin cleavage. The construction of the aforementioned plasmid, pGEX-2T/LIF, was achieved by standard techniques, and the plasmid was introduced into E.coli NM522 (Gough, J.A. and Murray, N.M., J.Mol.Biol 166: 1-19, 1983) by standard techniques.

Step 2: Expression and purification of glutathione S-transferase/LIF fusion protein.

In order to induce the expression of the glutathione S-transferase/LIF fusion protein, 10ml cultures of E.coli NM522 cells containing pGEX-2T/LIF were grown to logarithmic phase in Luria broth, and isopropyl β-D-thiogalactopyranoside added to a concentration of 0.1mM. After a further 4 hours growth, during which time the glutathione S-transferase/LIF gene is expressed, the cells were harvested by centrifugation and resuspended in 1ml mouse tonicity phosphate-buffered saline (MTPBS: 150mM NaCl, 16mM Na_2HPO_4, 4mM NaH_2PO_4, pH7.3). Cells were lysed on ice by mild sonication and after adding Triton X-100 to 1% cell debris was removed by centrifugation (10,000g, 5 min, 4°C). The clarified supernatant was mixed at room temperature in a 50ml polypropylene tube on a rotating platform with 200µl 50% glutathione-agarose beads (sulphur linkage, Sigma). (Before use, beads were pre-swollen in MTPBS, washed twice in the same buffer and stored in MTPBS at 4°C as a 50% solution v/v). After absorption (5 min.), beads were collected by centrifugation (500g, 1 min) and washed three times in MTPBS/Triton X-100. The glutathione S-transferase/LIF fusion protein was then eluted by competition with free glutathione: beads were incubated with 100µl 50mM Tris. HCl, 5mM reduced glutathione (pH 7.5) for 2 min at room temperature and the beads removed by centrifugation. The elution step was performed twice, and the two 100µl aliquots of eluate pooled.

100µl of the glutathione S-transferase/LIF fusion protein was treated with thrombin as described (Smith, D.B. and Johnson, K.S., supra).

1µl aliquots of the uncleaved and thrombin-cleaved glutathione S-transferase LIF fusion proteins were electrophoresed on a Pharmacia Phast Gel (8-25% polyacrylamide gradient gel). After staining with Coomassie Blue, a single major protein species of relative molecular weight ~46 kDa was revealed in the uncleaved preparation, and in the thrombin-cleaved preparation two major bands of ~26 kDa and ~20 kDa (corresponding to the expected sizes of the fusion protein (46 kDa), the glutathione S-transferase (26 kDa) and the LIF proteins (20 kDa) respectively). By estimating the mass of the Coomassie-stained bands on this gel, the yield of glutathione S-transferase/LIF protein from the original 10ml E.coli culture was estimated to be ~1.5µg.

Assays for differentiation-inducing activity and leukaemia-suppressive activity of both the glutathione S-transferase/LIF fusion protein and the thrombin cleaved LIF preparation, were performed using murine M1 cells (as described previously). Both preparations of LIF were determined to be biologically active, with specific activities in excess of $7x10^6$ units/mg.

EXAMPLE 8

The following sets out the steps used to determine whether the murine genome contains any other genes closely related to the gene encoding the sequence present in clone pLIF7.2b (Example 4) and the derivation of a map of the location of restriction endonuclease cleavage sites around the LIF gene.

The accompanying Figures relate to various steps of the method described below:

Figure 17: relates to step 1: hybridization of mouse DNA with a pLIF7.2b-derived probe under high and low stringency conditions. BALB/c liver DNA digested with the indicated restriction enzymes was probed for LIF gene sequences as described in Example 8, step 1. In the left hand panel, hybridization was at 65°C in 2 x SSC, and final washing at 65°C in 0.2 x SSC. In the right hand panel, hybridization and washing were both in 6 x SSC at 65°C. In the left hand panel, linear pLIF7.2b plasmid DNA (5.6 kb) was included at an amount equivalent to 10 and 1 copies per haploid mouse genome (400 pg and 40 pg respectively) assuming a molecular weight for the haploid mouse genome of 3×10^9 bp (Laird, C.D. Chromosoma, 32: 378-406, 1971). The sizes of the hybridizing genomic fragments are indicated.

Figure 18: relates to step 2: Hybridization of mouse DNA cleaved with various restriction endonucleases, singly and in pair-wise combinations, with a mouse LIF probe. Digested DNAs were electrophoresed on 0.8% agarose gels and hybridized with the pLIF7.2b cDNA fragment as described in Example 8, step 1, under high stringency conditions.

Figure 19: relates to step 2: Restriction endonuclease cleavage map of the murine LIF gene. The region of chromosomal DNA containing sequences corresponding to the cDNA clone pLIF7.2b is indicated by a box. The restriction sites given above (Eco R5) and below (Xba I, Bam HI, Pst I and Stu I) the central line have not been oriented with respect to the central line. The relative location of sites within the complex below the line is as indicated.

Figure 20: relates to step 3: chromosomal assignment of the murine LIF gene. In lane 1 BALB/c mouse embryo DNA was electrophoresed, in lane 2, DNA from Chinese hamster ovary cells and in lanes 3-8 DNA from the hybrid clones I-18A HAT, I-18A-2a- 8AG; EBS 58; EBS 11; EBS 4; and I-13A-1a-8AG (Cory, S. et al, EMBO J. 2: 213-216, 1983). The murine chromosome content of these hybrids is given in Cory, S. et al, (EMBO J. 2: 213-216, 1983). All DNAs were digested with Bam HI. The position of the 3 kbp Bam HI fragment bearing the murine LIF gene is indicated.

Step 1: Determination of the number of LIF-related genes in the murine genome.

In view of the data (Example 1) that medium conditioned by Krebs II cells contains two biochemically separable but functionally similar factors capable of inducing the differentiation of M1 cells (LIF-A and LIF-B) we wished to determine how many genes there are in the murine genome related to the species we have purified and cloned. Southern blots of murine genomic DNA digested with various restriction endonucleases were therefore hybridized with a probe derived from pLIF7.2b under conditions of both high and low stringency.

20 µg aliquots of high molecular weight genomic DNA from BALB/c mouse livers were digested to completion with various restriction endonucleases, fractionated by electrophoresis in 0.8% agarose gels and transferred to nitrocellulose. Prior to hybridization, filters were incubated for several hours at 65 °C in either 6 x SSC (low stringency) or 2 x SSC (high stringency) (SSC = 0.15M NaCl, 0.015M sodium citrate), 0.2% Ficoll, 0.2% polyvinyl-pyrollidone, 0.2% bovine serum albumin, 2mM sodium pyrophosphate, 1mM ATP, 50 µg/ml denatured salmon sperm DNA and 50 µg/ml E.coli tRNA. Hybridization was in the same solution containing 0.1% SDS, at 65 °C for 16-18 hours. Filters were then washed in either 6 x SSC, 0.1% SDS at [illegible] °C (low stringency) or in 2 x SSC, 0.1% SDS at 65 °C, followed by 0.2 x SSC, 65 °C (high stringency) as detailed in the legend to Figure 17. The hybridization probe used was as previously disclosed the approx.750 bp Eco RI -Hind III fragment spanning the cDNA insert of pLIF7.2b radiolabelled to a specific activity of approx.4 x 10^8 cpm/µg by nick-translation and included in the hybridization at a concentration of approx.2 x 10^7 cpm/ml.

In mouse liver DNA (Figure 17) as well as in DNA from Krebs II cells, LB3 T cells and WEHI265 monocytic cells (not shown) the LIF probe detected unique Eco RI, Bam HI, and Hind III fragments of approximately 11, 3 and 13 kbp respectively. The same pattern of hybridization was evident at both high (2 x SSC, 65 °C) and low stringency (6 x SSC, 65 °C) (Figure 17); under low stringency hybridization and washing conditions no additional bands were evident. Similarly, unique hybridizing fragments were also detected in Pst I, Stu I, Sac I, Eco R5 and Bgl II-digested genomic DNA (Figure 17). Moreover, in the experiment illustrated in Figure 16, pLIF7.2b plasmid DNA was included at a concentration equivalent to 10 and 1 copies per haploid mouse genome (tracks 1 and 2); the hybridization intensity of the genomic LIF sequences was not significantly different from the intensity of the unique gene standard (track 2).

Taken together, the foregoing data indicate that the LIF gene is unique in the murine genome, with no close relatives. Thus the two species of LIF are not likely to be products of different genes, but rather are more likely to represent post- transcriptional or post-translational variants of the same gene product.

Step 2: Derivation of a restriction endonuclease cleavage map of the murine LIF gene.

In order to determine the disposition of restriction endonuclease cleavage sites in and around the murine LIF gene, and thus provide a molecular fingerprint of this gene, DNA from mouse livers or from Krebs ascites tumour, cells was digested with various restriction endonucleases and subjected to Southern blot analysis as described in Step 1 (under high stringency hybridization and washing conditions). Examples of such experiments are shown in Figure 18. Analysis of the size of the digestion products enables one to generate a map of the location of each cleavage site around the LIF gene (Figure 19).

Step 3: Chromosome assignment of the murine LIF gene.

To determine the chromosome on which the mouse LIF gene is located, DNA was examined from six mouse-Chinese hamster ovary somatic cell hybrid cell lines which retain various mouse chromosomes (Cory, S. et al, EMBO J. 2: 213-216, 1983; Francke, U. et al, Cytogenet.Cell.Genet. 19: 57-84, 1977). Southern blot analysis performed as described in Step 1 (using high stringency hybridization and washing conditions) indicated that the 3 kbp Bam HI fragment containing the murine LIF gene was absent from all of the hybrids (Figure 20). Since chromosome 11 is the only mouse chromosome not retained in any of these lines (Cory, S. et al, EMBO J. 2: 213-216, 1983), a characteristic of mouse-Chinese hamster hybrids (Francke, U. et al, Cytogenet.Cell.Genet. 19: 57- 84, 1977), it is likely that the LIF gene is on this chromosome. On the same basis, the murine GM-CSF gene (Barlow, D.P. et al, EMBO J. 6: 617-623, 1987) and Multi-CSF gene (Ihle, J.N. and Kozak, C.A., National Cancer Institute, Frederick Cancer Research Facility, Annual Report, 1984) have also been assigned to chromosome 11, an assignment confirmed by genetic linkage studies (Barlow, D.P. et al, EMBO J. 6: 617-623, 1987).

EXAMPLE 9.

The following example sets out the steps used to establish conditions under which the cloned murine LIF cDNA can be used to identify by hybridization a human gene or mRNA or recombinant DNA clones containing human LIF-encoding sequences.

The accompanying Figure (Figure 21) relates to Step 2 of the method described below: hybridization of ^{32}P- labelled fragment of cDNA from clone pLIF7.2b under various conditions to genomic DNA of both mouse and human origin. In each case, track 1 contains 15µg murine (LB3) DNA and track 2 and 3 contain 15µg human DNA (from the cell lines Raji or Ramos, respectively. The hybridization and washing conditions applied to each filter are described in Step 2. The approximately 10 kbp Eco RI fragment containing the murine LIF gene, and the approximately 9 kbp Eco RI fragment containing the human homologue, are arrowed. Molecular weight standards are given at the left. Two different autoradiographic exposures (16 hours and 62 hours) are shown.

Step 1: Preparation and Radioactive Labelling of a Fragment of cDNA from pLIF7.2b

pLIF7.2b plasmid DNA was amplified by growth in E.coli MC1061 (Casadaban, M. and Cohen, S J.Mol.Biol. 138:179-207, 1980), extracted from the E.coli cells by standard procedures (Maniatis, J. et al. Molecular Cloning, Cold Spring Harbor, New York, 1982) and purified by CsCl-gradient centrifugations pLIF7.2b plasmid DNA so purified was cleaved with the restriction endonucleases Eco RI and Hind III to release a cDNA-containing fragment of ~770 bp from the vector (pJL3), which was resolved from vector sequences by electrophoresis through a 1.5% agarose gel.

200 ng of pLIF7.2 cDNA fragment thus purified was radioactively labelled to a specific activity of approximately $3x10^8$ cpm/µg by nick-translation (Rigby, P.W.J., Dieckmann, M., Rhodes, C. and Berg, P. J.Mol.Biol. 113:237-251, 1977) in a reaction containing 100µCi [α^{32}P]-dATP. The radioactively labelled cDNA fragment was purified from unincorporated label by precipitation in the presence of 1M sodium perchlorate and 33% isopropanol.

Step 2: Hybridization of Southern Blots of Mouse and Human Genomic DNA with a pLIF7.2b cDNA Probe

High molecular weight genomic DNA (15µg) from the murine T cell line LB3 (lane 1) and from two human B cell lines (Raji and Ramos; lanes 2 and 3) cleaved with the restriction endonuclease Eco RI was electrophoresed through a 0.8% agarose gel and transferred to nitrocellulose using standard techniques (Southern, E. M., J.Mol.Biol. 98:503-517, 1975). Five identical Southern blots were prepared containing each of these three DNAs. Before hybridization, filters were incubated for several hours at 55°C in either 0.9M NaCl, 0.09M sodium citrate, 0.2% Ficoll, 0.2% polyvinyl-pyrollidone, 0.2% bovine serum albumin, 50µg ml heat denatured salmon sperm DNA, 50 µg/ml E.coli tRNA, 0.1mM ATP and 2mM sodium pyrophosphate (filters a, b, c, d) or in 0.3M NaCl, 0.03M sodium citrate, with the same additional components (filter e). Hybridization with the ^{32}P-labelled pLIF7.2b cDNA prepared in Step 1 was performed in the same solutions as the prehybridization, containing in addition 0.1% sodium dodecyl sulphate, at 55°C (filters a, b, c), or 65°C (filters d, e) for 16 hours. The ^{32}P-labelled cDNA was denatured by boiling prior to hybridization and was included in the hybridization reaction at ~10^7 cpm/ml.

After hybridization the filters were washed in either 0.9M NaCl, 0.09M sodium citrate, 0.1% sodium dodecyl sulphate at 55°C (filter a) 60°C (filter b) or 65°C (filters c, d) or in 0.3M NaCl, 0.03M sodium citrate, 0.1% sodium dodecyl sulphate at 65°C (filter e). After exhaustive washing, filters were autoradiographed at -70°C using Kodak XAR-5 film and two intensifying screens. Figure 21 shows the results of such an experiment, in which two of the hybridization/washing regimes described (filters d and e) allowed both the murine LIF gene (present on an approximately 10 kbp Eco RI fragment) and the human LIF gene (present on an approximately 9 kbp Eco RI fragment) to be detected above residual background non-specific hybridization. All other conditions tested gave rise to an unacceptably high level of background hybridization (filters, a, b, c).

EXAMPLE 10

The following example sets out the steps used to obtain a cloned human gene homologous to murine LIF.

The accompanying Figures relate to various steps of the method described below. In the Figures:

Figure 22: relates to step 1 of the cloning of the human LIF gene: Detection of the LIF gene by Southern blot hybridization using a mouse LIF cDNA probe. Genomic DNA from the human cell line RAMOS was digested with the indicated restriction endonucleases and hybridized under the conditions described in Example 19, step 1, with a mouse cDNA fragment derived from pLIF7.2b.

Figure 23: relates to step 1 of the cloning of the human LIF gene: detection of the LIF gene by Southern blot hybridization using a mouse LIF cDNA probe under a variety of hybridization conditions. Genomic DNA from the human cell line RAMOS (H) or mouse liver DNA (M) digested with the restriction endonuclease Bam HI were hybridized with a murine LIF cDNA probe (pLIF7.2b) under a variety of conditions of hybridization and washing. The temperatures and concentrations of SSC used for hybridization are indicated on the top line and the conditions of washing on the second line (see Example 10, step 1).

Figure 24: relates to step 2 of the cloning of the human LIF gene: restriction endonuclease cleavage of three candidate LIE gene clones. 1 µg of DNA from λ phage of the clones λHGLIF1, λHGLIF2 and λHGLIF3 was digested with Sal I, Bam HI or Pst I, electrophoresed on an 0.8% agarose gel (left hand panel), transferred to nitrocellulose and hybridized (as disclosed previously) with pLIF7.2 cDNA. The approximate sizes of the hybridizing fragments are indicated.

Figure 25: relates to step 3 of the cloning of the human LIF gene: The nucleotide sequence of the mRNA- synonymous strand of a 1.3kbp segment of λHGLIF1 spanning the human LIF gene (H) is presented in a 5' to 3' orientation. The corresponding nucleotide sequence of the murine LIF mRNA (M) derived from cDNA clones pLIF7.2b, pLIFNK1 and pLIFNK3 is aligned beneath the human gene and given in lower case letters. Identities between the mouse and human sequences are indicated with asterisks. The presumed N-terminal residue of mature human LIF, by analogy with mouse LIF, is designated as +1.

Figure 26: relates to step 3 of the cloning of the human LIF gene: amino acid sequence of human LIF and comparison with mouse LIF. The amino acid sequence of mature murine LIF (M) as determined by direct amino acid sequencing, and analysis of the cDNA clones pLIF7.2b, pLIFNK1 and pLIFNK3 is listed on the top line, with the corresponding sequence of human LIF (H), as deduced from the sequence of λHGLIF1, listed below. Identities are indicated with dashes; differences are indicated by designation of the amino acid.

Figure 27: relates to step 4 of the cloning of the human LIF gene: restriction endonuclease cleavage map of the human LIF gene. The exons of the human gene homologous with pLIF7.2b (Figure 25) are indicated as boxes. The direction of transcription of the gene is indicated by the arrow below the line.

Step 1: Detection of the human LIF gene with a mouse probe.

A method has been previously disclosed for using a radioactively labelled fragment of the mouse LIF cDNA clone pLIF7.2b as a hybridization probe to detect the human LIF gene. Figure 22 demonstrates that this method allows the human LIF gene to be detected in human genomic DNA digested with a variety of restriction endonucleases. Analyses such as this, and other gels not shown, revealed the sizes of DNA fragments generated by a variety of restriction endonucleases on which the human LIF gene is located. Such data is of importance in subsequent steps of this example, not only to establish conditions under which the mouse probe can be used as a hybridization probe to detect the human, but also to provide diagnostic restriction mapping data to aid in identifying human genomic LIF clones.

A high degree of homology between the mouse and human LIF sequences was apparent when mouse and human genomic DNA digested with Bam HI was hybridized with a mouse LIF cDNA probe under a variety of conditions of hybridization and washing (Figure 23). As the stringency of hybridization and washing was raised, so the background smear was reduced, revealing a unique fragment of ~3 kbp hybridizing with the mouse probe. Significantly, the human gene retained substantial hybridization even at 65 °C in 0.2 x SSC.

Step 2: Screening of a human genomic library and isolation of a clone containing the LIF gene.

A library of human genomic DNA, partially digested with Sau 3A and ligated into the lambda phage cloning vector EMBL3A, was screened for LIF gene-containing clones by hybridization with the mouse cDNA as a probe. The fragment of LIF cDNA was radioactively labelled and the conditions of hybridization were as disclosed previously (Example 4). Briefly, phage plaques representing the genomic library were grown at a density of ~50,000 plaques per 10 cm petri dish and transferred to nitrocellulose as described (Maniatis, T. et al, Molecular Cloning, Cold Spring Harbor, 1982). Prior to hybridization, filters were incubated for several hours at 65 °C in 6 x SSC (SSC = 0.15M NaCl, 0.015M sodium citrate), 0.2% Ficoll, 0.2% polyvinyl-pyrollidone, 0.2% bovine serum albumin, 2mM sodium pyrophosphate, 1mM ATP, 50 µg/ml denatured salmon sperm DNA and 50 µg/ml E.coli tRNA. Hybridization as in the same solution containing 0.1% SDS, at 65 °C for 16-18 hours. The LIF cDNA fragment, radioactively labelled by nick-translation using [α^{32P}] dATP to a specific activity of ~2 x 10^8 cpm/µg was included in the hybridization at a concentration of ~2 x 10^6 cpm/ml. After hybridization, filters were extensively washed in 6 x SSC, 0.1% sodium dodecyl sulphate at 65 °C and then autoradiographed. Plaques positive on duplicate filters were picked and rescreened at lower density, as before.

Three clones were thus identified and purified: λHGLIF1, 2 and 3. In order to determine the relationship between these clones and to determine which, if any, contain the human LIF gene, DNA was prepared from each clone and digested with these restriction endonucleases: Sal I which liberates the entire segment of cloned genomic DNA and Bam HI and Pst I which cleave in and around the LIF gene to generate characteristic fragments of ~3 kbp and 1.8 kbp and 0.6 kbp respectively (as determined above). After digestion of the recombinant phage DNAs and resolution by electrophoresis on agarose gels (Figure 24, left hand panel), the DNA was transferred to nitrocellulose and hybridized with the mouse LIF cDNA probe (under the conditions outlined above) to reveal the fragments containing the LIF gene (Figure 24, right hand panel). This analysis revealed that

(a) all three clones appeared to be identical;

(b) all contain an ~9 kbp segment of genomic DNA containing a region homologous with the mouse LIF probe;

(c) the region of homology with the mouse cDNA is present on a 3 kbp Bam HI fragment and on 1.8 and 0.6 kbp Pst I fragments, characteristics of the human LIF gene (see above). Thus it was concluded that all three clones contain a segment of chromosomal DNA encompassing the human LIF gene.

Step 3: Determination of the nucleotide sequence of the human LIF gene.

The ~3 kbp Bam HI fragment of λHGLIF1 shown above to hybridize with the mouse LIF cDNA probe was recloned into the plasmid vector pEMBL8+, giving rise to clone pHGLIFBamI, and subjected to nucleotide sequence analysis. Nucleotide sequencing was performed by the dideoxy chain termination method (Sanger, F et al, Pro.Natl.Acad.Sci.USA. 74: 5463-5467, 1977) using alkaline denatured double-stranded plasmid DNA as template, a variety of oligonucleotides complementary to sequences within the gene as primers, and using both the Klenow fragment of E.coli DNA polymerase I and the avian myeloblastosis virus reverse transcriptase as polymerases in the sequencing reactions.

The entire nucleotide sequence of the Bam HI fragment spanning the LIF gene (2840bp) was determined, of which 1297bp is shown in Figure 25. Alignment of this sequence with the murine LIF mRNA sequence revealed that the sequences encoding the mature human LIF protein are present on two exons separated by an intron of 693bp (Figure 25). For the region encoding the mature protein there is a high degree of homology between the two species at both the nucleotide and the amino acid sequence level. Within exon 1 there is 88% nucleic acid sequence homology (114/129 residues compared) and 91% amino acid sequence homology (39/43) for the region encoding the mature protein (position 58-186). Exon 2 is somewhat less homologous, 77% at the nucleotide level (318/411) and 74% at the amino acid level (101/136) within the coding region. Considering the mature protein as a whole, mouse and human LIF sequences determined here are identical at 140 of 179 positions (78%) with no insertions or deletions

(Figure 26). Moreover, many of the differences are highly conservative substitutions (Lys:Arg, Glu:Asp and Leu:Val:Ala).

5′ of the codon for the N-terminal proline residue of mature LIF, (position 58 in Figure 25) the human gene is homologous with the murine LIF mRNA sequence through a region encoding most of the hydrophobic leader. However, the entire leader is not encoded on this exon, since the mRNA and gene sequences diverge at a typical RNA splice site (TCCCCAG) (Mount, S.M., Nucleic Acids Res. 10:459-472, 1982). The exon specifying the 5′ untranslated region and the first residues of the leader is not present within 1097 bp 5′ of this splice site. In the mouse LIF gene, the exon specifying the first 6 amino acid residues of the hydrophobic leader is located ~1.5kbp 5′ of the analogous splice site.

Step 4: Derivation of a restriction endonuclease cleavage map of the human LIF gene.

In order to determine the disposition of the restriction endonuclease cleavage sites in and around the human LIF gene, and thus provide a molecular fingerprint of this gene, human genomic DNA (from the RAMOS cell line) was digested with various restriction endonucleases singly and in pair-wise combinations and subject to Southern blot analysis as described in Example 8, except that the probe used was the 3 kbp Bam HI fragment derived from pHGLIFBamI described in Step 3 above and radio-labelled by nick-translation. Analysis of the data so-derived (not shown), as well as that shown in Figure 22 and derived from analysis of λHGLIF1 and pHGLIFBamI, gave rise to the restriction endonuclease cleavage map shown in Figure 27.

EXAMPLE 11

The following example details modifications made to the cloned human LIF gene in order to allow expression in yeast cells and determination of the biological and biochemical properties of the recombinant human LIF so derived.

In the Figures,

Figure 28: relates to step 1: oligonucleotides used to modify the human LIF gene for incorporation into YEpsec1. Oligonucleotide (a) corresponds to the 5′ end of the coding region (residues 31 to 69 in Figure 25). Oligonucleotide (b) corresponds to the middle of the coding region (residues 163 to 186 and 880 to 903 in Figure 25). The portion of this oligonucleotide complementary to exon 1 is underlined with dashes, and that complementary to exon 2 with dots. Oligonucleotide (c) corresponds to the 3′ end of the coding region (from position 1279 in Figure 25). Oligonucleotides (a) and (b) introduce the indicated restriction endonuclease cleavage sites.

Figure 29: relates to step 1: nucleotide sequence of, and amino acid sequence encoded by, the synthetic human LIF cDNA derived by mutagene sis of the cloned human LIF gene. The Bam HI and Hind III cleavage sites introduced by oligonucleotides (a) and (c) (Figure 28) are indicated. The presumed N-terminal amino acid of mature LIF (by analogy with the mouse) is designated as +1.

Figure 30: relates to step 3: Induction of differentiation in colonies of M1 leukaemic cells by dilutions of purified native murine LIF (o------o) and conditioned medium from yeast cells containing the YEpsec1/HLIF recombinant induced with galactose (●_____●).Medium from uninduced yeast cultures containing the YEpsec1/HLIF recombinant (o_____o) was inactive. Mean data from replicate 7 day cultures is presented.

Figure 31: relates to step 4: competition of yeast-derived HLIF with native murine ^{125}I-LIF for binding to specific receptors on murine M1 cells. Dilutions of authentic native murine LIF-A (Example 1) (o_____o), recombinant murine LIF (●_____●) and conditioned medium from yeast cells containing the YEpsec1/HLIF construct uninduced (■_____■) and induced with galactose (□_____□) were tested for ability to compete for the binding of native ^{125}I-LIF-A to cellular receptors on murine M1 cells at 37°C, as disclosed previously.

Step 1: Modification of the human LIF gene for expression in yeast.

The isolation of a recombinant DNA clone containing the human LIF gene and the nucleotide sequence of the aforementioned gene has been previously disclosed (Example 10). The nucleotide sequence of 1297bp of DNA spanning the 2 exons encoding the mature human LIF protein is shown in Figure 25.

Murine recombinant LIF has previously been produced in yeast cells using the yeast expression vector, YEpsec1 (Example 5). This vector provides an N-terminal leader sequence derived from the killer toxin gene of Kluyveromyces lactis, transcribed from a galactose-inducible hybrid GAL-CYC promoter.

In order to express the protein encoded by the human LIF gene in this vector it was necessary to modify the gene in several ways. At the 5′ end of the region encoding the mature protein a cleavage site for the restriction endonuclease Bam HI was introduced to allow insertion in frame with the K.lactis leader and retain an appropriate signal peptidase cleavage site (Gly-Ser). The same modification as previously applied to the mouse cDNA (pLIF7.2b) was made here. In the middle, the 693 bp intervening sequence was removed, fusing the two exons in the same translational reading frame. At the 3′ end, a second translational stop codon was introduced immediately 3′ of the natural stop codon, followed by a Hind III site for insertion into YEpsec1. All of the modifications were achieved by oligonucleotide-mediated mutagenesis: the ~3kbp Bam HI fragment spanning the LIF gene was subcloned into the plasmid pEMBL8+, (Dente, L. et al. Nucleic Acids Res. 11:1645-1655, 1983) and single-stranded DNA prepared by F1 superinfection In vitro mutagenesis was performed as described (Nisbet, I.T. and Beilharz, M.W. Genet Anal. Techn 2 23 29. 1985), using oligonucleotides of 39, 48 and 39 bases respectively to modify the 5′ end, the middle and 3′ end of the gene as outlined above (see Figure 28). The nucleotide sequence of the modified human LIF coding region is given in Figure 29.

Step 2: Introduction of the YEpsec1/HLIF recombinant into yeast cells.

S.cerevisiae strain GY1+ (leu2 ura3 ade2 trp1 cir+; from G. Cesareni, EMBL Heidelberg) was transformed by the polyethylene glycol method (Klebe, R.J. et al, Gene 25: 333-341, 1983). Transformants were selected and maintained on synthetic minimal medium (2% carbon source, 0.67% yeast nitrogen base (Difco) supplemented with 50μg/ml of the required amino acids) under uracil deprivation. Recombinant HLIF was produced by either of two methods. Either (1), Ura+ transformants were grown to stationary phase in non-selective medium containing 2% galactose, and the medium assayed for LIF activity or (2). Ura+ transformants were grown to stationary phase in selective minimal medium containing 2% glucose Cells were then washed and resuspended in the same volume of selective minimal medium containing 2% ethanol and grown for 8 hours to overcome glucose repression. Transcription of the HLIF insert was then induced by diluting the cells (1:10) into synthetic minimal medium containing 2% galactose. Aliquots of culture supernatant were removed at various times after induction, filtered through Millipore filters (0.2μm) and assayed for LIF activity directly.

Step 3: Determination of the biological properties of yeast-derived HLIF.

In view of the high degree of sequence similarity between mouse and human LIF, the activity of yeast derived human LIF on murine M1 cells was assessed. Assays for differentiation-inducing activity and leukaemia-suppressive activity of yeast conditioned medium were performed in 1 ml cultures containing 300 murine M1 cells (provided by Dr M. Hozumi, Saitama Cancer Research Centre, Japan) in Dulbecco's Modified Eagle's medium with a final concentration of 20% foetal calf serum and 0.3% agar. Material to be assayed was added in serially diluted 0.1 ml volumes to the culture dish prior to the addition of the cell suspension in agar medium. Cultures were incubated for 7 days in a fully humidified atmosphere of 10% CO_2 in air. Cultures were scored using a dissection microscope at x35 magnification, scoring as differentiated any colonies with a corona of dispersed cells or composed wholly of dispersed cells. Morphological examination of colonies was performed by fixing the entire culture with 1 ml 2.5% glutaraldehyde then staining the dried cultures on microscope slides using acetylcholinesterase/Luxol Fast Blue/Haematoxylin.

Medium from galactose-induced cultures of yeast containing the human coding region in YEpsec1. but not from uninduced cultures of the same yeast cells, from cultures of non-transformed yeast, or yeast containing the vector YEpsec1 alone, was able to induce typical macrophage differentiation in cultures of M1 colonies (Figure 30). As the murine LIF, with increasing concentrations the yeast-derived human material also progressively reduced the number and size of M1 colonies developing. Comparison with purified native murine LIF indicated that the yeast conditioned medium contained up to 50,000 Units/ml of human LIF.

Step 4: Receptor binding specificity of yeast-derived human LIF.

Purified murine LIF-A (Example 1) was iodinated as disclosed previously. M1 cells were washed and resuspended at 2.5 x 10^6/50μl in Hepes-buffered RPMI medium containing 10% foetal calf serum. Cells in 50 μl aliquots were incubated with 200,000 cpm of ^{125}I-LIF-A (10μl in the same medium) and 10μl of control medium or serial two-fold dilutions of unlabelled pure murine LIF-A or conditioned medium from galactose-induced or uninduced cultures of yeast transformants containing the YEpsec1/HLIF construct.

Medium conditioned by galactose-induced yeast cells containing the YEpsec1/HLIF recombinant was able to compete for the binding of native murine ^{125}I-LIF-A to specific cellular receptors on murine M1 cells to the same extent as native and recombinant murine LIF-A, at 37°C. (Figure 31) and at 0°C (not shown). Medium from uninduced yeast cells and from yeast cells containing the vector YEpsec1 alone did not. Thus there appears to be a strong conservation of the receptor binding domain in murine and human LIFs, compatible with the high degree of primary amino acid sequence similarity.

Step 5: Purification, sequencing and iodination of yeast-derived human LIF.

Human LIF in medium conditioned by galactose-induced yeast cells containing the YEpsec 1/HLIF recombinant was purified using steps 2 and 4 of Example 1, except that the LIF activity binding to the DEAE-Sepharose CL-6B column and eluted with the salt gradient was pooled in step 2. Purified yeast-derived human LIF was radioiodinated by incubating 1μg of human LIF in 50μl of 0.2M sodium phosphate buffer, pH 7.2, with 1mCi of Na ^{125}I (2.7μl) and 5μl of 0.2mM ICl in 2M NaCl for 60 sec. ^{125}I-LIF was separated from unincorporated ^{125}I by passage of the reaction mixture through a column of Sephadex G-25M (Pharmacia) equilibrated in phosphate buffered (20mM, pH 7.4) saline (0.15M) containing 0.02% Tween 20 and 0.02% sodium azide. Human ^{125}I-LIF electrophoresed on a 8-25% gradient sodium dodecyl sulphate polyacrylamide gel as a single broad band with apparent molecular weight of 170,000. Human ^{125}I-LIF bound specifically to murine M1 cells and bone marrow cells confirming the ability of human LIF to bind to murine LIF receptors. Purified yeast-derived human LIF (approx. 10μg) was subjected to amino-terminal amino acid sequencing as described in Example 2 and gave a single sequence of:

Ile-Thr-Pro-Val-X-Ala ..... .

This is identical to the predicted amino acid sequence of human LIF (Figure 26) except that the sequence begins at the fourth amino acid compared to the start of the murine sequence (see Example 2) which is:

Pro-Leu-Pro-Ile-Thr-Pro-Val-Asn-Ala ..... .

This indicates that the purified yeast-derived human LIF is missing the corresponding first three amino acids of the mouse sequence but is still biologically active and still able to bind to the murine LIF receptor. The first three amino acids thus appear to be dispensable for the biological activity of LIF.

EXAMPLE 12

The following example sets out the steps used to identify and partially purify a putative native human LIF molecule.

The accompanying Figures relate to various steps of the method described below. In the Figures:

Figure 32: relates to step 2 of the identification of a putative human LIF: the ability of different dilutions of medium conditioned by the human bladder carcinoma cell line 5637 (ATCC No. HTB9) (- □ -) crude or (- + -) DEAE non-binding fraction or of native murine LIF-A (- o -) to compete for the binding of murine ^{125}I-LIF-A to cellular receptors on murine peritoneal cells. The inability of human G-CSF (- ■ -) to compete for binding is also shown (undiluted = 5μg/ml).

Figure 33: relates to step 3 of the identification of a putative human LIF: the fractionation of medium conditioned by the human bladder carcinoma cell line 5637 on a column of DEAE- Sepharose CL-6B, eluted exactly as for the previously described fractionation of murine LIF-A (Example 1), and the ability of individual fractions from this fractionation to induce the formation of differentiated colonies of murine M1 cells. Panel A shows the salt gradient, Panel B shows the fractionation of 5637 conditioned medium; Panel C shows the fractionation of Krebs II cell conditioned medium for comparison.

Figure 34: relates to step 3 of the identification of a putative human LIF: the fractionation of medium conditioned by the human bladder carcinoma cell line 5637 on a column of lentil-lectin Sepharose 4B eluted as previously described for the fractionation of murine LIF-A (Example 1) and the ability of individual fractions from this fractionation to induce the formation of differentiated colonies of murine M1 cells. Panel A shows the gradient of α-methyl-D-mannopyrannoside, Panel B shows the fractionation of 5637 conditioned medium and Panel C shows the fractionation of Krebs II conditioned medium for comparison.

Step 1: Medium conditioned by several human cell lines were assayed for their capacity to induce the differentiation and inhibit the proliferation of M1 murine myeloid leukaemic cells in semi-solid agar

cultures (as described in Example 5, Step 4 of the present application). Several cell lines produced such an activity, of which the bladder carcinoma cell line 5637 produced the highest levels. However, the 5637 cell line has been shown previously to produce human G-CSF (Nicola, N.A. et al, Nature 314: 625-628. 1985; Welte, K. et al, Proc.Natl.Acad.Sci. USA 82: 1526-1530, 1985) which is also active in inducing differentiation of M1 cells (Tomida, M. et al, FEBS Lett. 207: 271-275, 1986; Neckers, L.M. and Pluznik, D.H. Exp.Hematol. 15: 700-703, 1987). To confirm that 5637 cells produced authentic human LIF (in addition to G-CSF), 5637-conditioned medium was further subjected to Steps 2 and 3 below.

Step 2: Medium conditioned by 5637 cells was concentrated 20- fold and tested for its ability to compete for the binding of native murine ^{125}I-LIF-A to specific cellular receptors on murine peritoneal cells This competition binding assay was as disclosed in Step 5 of Example 5 in the present application. 5637 cell-conditioned medium contained activity capable of competing for the binding of murine ^{125}I-LIF-A to cellular receptors and this activity was concentrated in the DEAE non-binding fraction of 5637 CM (LIF-A) (Figure 32). Since human and murine G-CSF do not compete, even at very high concentrations, for ^{125}I-LIF-A binding sites, this establishes the presence in 5637-conditioned medium of a homologous human LIF activity capable of recognizing specifically the murine LIF receptor. This indicates strong conservation of the murine and human LIFs in their receptor binding domain, compatible with the high degree of primary amino acid sequence homology.

Step 3: Medium conditioned by human bladder carcinoma 5637 cells (two litres in 10% v/v foetal calf serum) was concentrated to 40 ml and chromatographed sequentially on DEAE-Sepharose CL-6B and lentil-lectin-Sepharose 4B as described previously for murine LIF from Krebs II ascites cell-conditioned medium. M1 differentiation-inducing activity from 5637 cells (putative human LIF) chromatographed in a very similar fashion to murine LIF on DEAE-Sepharose, with some activity not binding to the column (LIF-A), while the remainder bound and was eluted during the salt gradient (LIF-B) (Figure 33). Similarly, the putative human LIF behaved like murine LIF on lentil-lectin-Sepharose chromatography with a proportion of activity binding to the column indicating the presence of mannose-containing carbohydrates on the glycoprotein (Figure 34). Thus by its cross-reactivity in inducing murine M1 cell differentiation, its ability to recognize specifically the murine LIF cellular receptor, and its biochemical fractionation characteristics, the human activity in 5637 cell conditioned media meets the criteria of the native human analogue of murine LIF.

Native human LIF from 5637 cell conditioned medium was purified by steps 2 and 4 of Example 1, pooling the non-binding LIF activity from step 2 and the binding LIF activity from step 4. This pooled LIF activity was fractionated by reverse-phase HPLC as for step 5 of Example 1 except that a Brownlee RP300 C8 column was used twice, first using a gradient from 0-60% CH_3CN in 0.1% TFA and then using a gradient from 45-55% CH_3CN in 0.1% TFA. In the second gradient human LIF eluted at 50% CH_3CN and when electrophoresed on 8-25% gradient sodium dodecyl sulphate polyacrylamide gels showed a major silver staining band of apparent molecular weight 73,000. Native purified human LIF was radioiodinated as described in step 5 of Example 10 and bound specifically to murine M1 cells and mouse bone marrow cells as described for yeast-derived human ^{125}I-LIF (step 5 of Example 11).

Claims

Claims for the following Contracting States : AT, BE, CH, LI, DE, FR, GB, IT, LU, NL, SE

1. A polypeptide having leukaemia inhibitory factor (LIF) activity, characterised in that it is capable of suppressing the proliferation of M1 myeloid leukaemic cells, it comprises the amino acid sequence set forth in Figures 15, 26 or 29 either completely or in part and in that it is capable of competing with a polypeptide of said sequence for binding to specific cellular receptors on M1 cells or murine or human macrophages.

2. A polypeptide having leukaemia inhibitory factor (LIF) activity, characterised in that it is capable of suppressing the proliferation of M1 myeloid leukaemic cells, it is encoded by a nucleotide sequence which is capable of hybridising under conditions of 6xSSC, 65 °C or conditions of higher stringency, to a sequence of a LIF encoding insert selected from the LIF-encoding inserts of clones pLIF7.2b. pLIFNK1, pLIFNK3, and pHGLIFBamI as defined in Figures 10, 11, 15 or 25 and in that it is capable of competing with a polypeptide of the amino acid sequence set forth in Figures 15, 26 or 29 for binding to specific cellular receptors on M1 cells or murine or human macrophages.

3. A polypeptide having leukaemia inhibitory factor (LIF) activity characterised in that:
   (a) it is capable of suppressing the proliferation of M1 myeloid leukaemic cells;

cultures (as described in Example 5, Step 4 of the present application). Several cell lines produced such an activity, of which the bladder carcinoma cell line 5637 produced the highest levels. However, the 5637 cell line has been shown previously to produce human G-CSF (Nicola, N.A. et al, Nature 314: 625-628. 1985; Welte, K. et al, Proc.Natl.Acad.Sci. USA 82: 1526-1530, 1985) which is also active in inducing differentiation of M1 cells (Tomida, M. et al, FEBS Lett. 207: 271-275, 1986; Neckers, L.M. and Pluznik, D.H. Exp.Hematol. 15: 700-703, 1987). To confirm that 5637 cells produced authentic human LIF (in addition to G-CSF), 5637-conditioned medium was further subjected to Steps 2 and 3 below.

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Native human LIF from 5637 cell conditioned medium was purified by steps 2 and 4 of Example 1, pooling the non-binding LIF activity from step 2 and the binding LIF activity from step 4. This pooled LIF activity was fractionated by reverse-phase HPLC as for step 5 of Example 1 except that a Brownlee RP300 C8 column was used twice, first using a gradient from 0-60% CH_3CN in 0.1% TFA and then using a gradient from 45-55% CH_3CN in 0.1% TFA. In the second gradient human LIF eluted at 50% CH_3CN and when electrophoresed on 8-25% gradient sodium dodecyl sulphate polyacrylamide gels showed a major silver staining band of apparent molecular weight 73,000. Native purified human LIF was radioiodinated as described in step 5 of Example 10 and bound specifically to murine M1 cells and mouse bone marrow cells as described for yeast-derived human ^{125}I-LIF (step 5 of Example 11).

Claims

Claims for the following Contracting States : AT, BE, CH, LI, DE, FR, GB, IT, LU, NL, SE

1. A polypeptide having leukaemia inhibitory factor (LIF) activity, characterised in that it is capable of suppressing the proliferation of M1 myeloid leukaemic cells, it comprises the amino acid sequence set forth in Figures 15, 26 or 29 either completely or in part and in that it is capable of competing with a polypeptide of said sequence for binding to specific cellular receptors on M1 cells or murine or human macrophages.

2. A polypeptide having leukaemia inhibitory factor (LIF) activity, characterised in that it is capable of suppressing the proliferation of M1 myeloid leukaemic cells, it is encoded by a nucleotide sequence which is capable of hybridising under conditions of 6xSSC, 65 °C or conditions of higher stringency, to a sequence of a LIF encoding insert selected from the LIF-encoding inserts of clones pLIF7.2b, pLIFNK1, pLIFNK3, and pHGLIFBamI as defined in Figures 10, 11, 15 or 25 and in that it is capable of competing with a polypeptide of the amino acid sequence set forth in Figures 15, 26 or 29 for binding to specific cellular receptors on M1 cells or murine or human macrophages.

3. A polypeptide having leukaemia inhibitory factor (LIF) activity characterised in that:
   (a) it is capable of suppressing the proliferation of M1 myeloid leukaemic cells;

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(b) it comprises the amino acid sequence set forth in Figures 15, 26 or 29 either completely or in part;
(c) it is capable of competing with a polypeptide of said sequence for binding to specific cellular receptors on M1 cells or murine or human macrophages; and
(d) it is encoded by a nucleotide sequence which is capable of hybridising under conditions of 6xSSC, 65 °C or conditions of higher stringency, to a sequence of a LIF encoding insert selected from the LIF-encoding inserts of clones pLIF7.2b, pLIFNK1, pLIFNK3, and pHGLIFBamI as defined in Figures 10, 11, 15 or 25.

4. A polypeptide having leukaemia inhibitory factor (LIF) activity, characterised in that it is capable of suppressing the proliferation of M1 myeloid leukaemic cells, it comprises the amino acid sequence set forth in Figures 15, 26 or 29 either completely or in part or a sequence which has at least 78% amino acid homology therewith, and in that it is capable of competing with a polypeptide of said sequence for binding to specific cellular receptors on M1 cells or murine or human macrophages.

5. A polypeptide having leukaemia inhibitory factor (LIF) activity which is capable of suppressing the proliferation of M1 myeloid leukaemic cells and which comprises the amino acid sequence set forth in Figures 15, 26 or 29.

6. A method of purifying a glycosylated or non-glycosylated LIF polypeptide as defined in claim 1, which comprises (a) chromatographing LIF in crude form on an anion exchange column, and eluting LIF by an increasing salt gradient, (b) chromatographing the product of step (a) on a lectin affinity column, said lectin having an affinity for mannose, and eluting LIF with a mannose derivative, (c) chromatographing the product of step (b) on a cation exchange column and eluting LIF with an increasing salt gradient, (d) chromatographing the product of step (c) under HPLC conditions on a reverse phase column and eluting LIF with an increasing gradient of acetonitrile.

7. The method of claim 6, wherein the LIF in crude form comprises a Krebs II ascites tumour cell conditioned medium, and murine LIF is purified from said medium.

8. The method of claim 6, wherein the LIF in crude form comprises human bladder carcinoma cell line 5637 (ATCC deposit No. HTB9) conditioned medium, and human LIF is purified from said medium.

9. A recombinant DNA molecule comprising a nucleotide sequence which codes for a LIF polypeptide as defined in any one of claims 1 to 5.

10. A recombinant DNA molecule comprising a nucleotide sequence as set forth in Figures 10, 15 (bases 1-630), 25 or 29 (bases 1-543) completely or in part, which encodes a polypeptide capable of suppressing the proliferation of M1 myeloid leukaemic cells and competing with a polypeptide having an amino acid sequence set forth in Figures 15, 26 or 29 for binding to specific cellular receptors on M1 cells or murine or human macrophages.

11. A recombinant DNA molecule comprising a nucleotide sequence which codes on expression in a suitable host cell for a LIF polypeptide as defined in claim 1, wherein said nucleotide sequence hybridizes under conditions of 6xSSC, 65 °C or conditions of higher stringency, to a sequence of a LIF encoding insert selected from the LIF-encoding inserts of clones pLIF7.2b, pLIFNK1, pLIFNK3, and pHGLIFBamI as defined in Figures 10, 11, 15 or 25.

12. A recombinant DNA molecule comprising a nucleotide sequence which codes on expression in a suitable host cell for a LIF polypeptide as defined in claim 1, wherein the nucleotide sequence codes on expression in a eukaryotic host for a polypeptide which comprises a mature polypeptide which is at least 78% homologous with a mature polypeptide amino acid sequence selected from those set forth in Figures 15, 26 and 29.

13. The molecule of claim 12, wherein the mature polypeptide has full homology with the amino acid sequence set forth in Figure 29.

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14. The molecule of any one of claims 9 to 13, further comprising an origin of replication, whereby said molecule is rendered suitable for use as a cloning vector.

15. The molecule of claim 14, further comprising a promoter sequence operably linked to said nucleotide sequence whereby said molecule is rendered suitable for use of an expression vector.

16. A host cell containing a recombinant DNA molecule according to claim 15.

17. A method of producing a LIF polypeptide as defined in claim 1 which comprises: (a) providing host cells containing a molecule according to claim 14, (b) cultivating said cells under conditions conducive to expression of said polypeptide; and (c) recovering said polypeptide.

18. The method of claim 17, wherein the polypeptide comprises a mature polypeptide which is at least 78% homologous with an amino acid sequence selected from those set forth in Figures 15, 26 and 29

19. The method of claim 17, wherein the mature polypeptide has full homology with the amino acid sequence set forth in Figure 29.

20. The method of claim 17, wherein the polypeptide is glycosylated by the host cell.

21. The method of claim 17, wherein the host cell is a yeast cell.

22. The method of claim 17, wherein the promoter is a galactose inducible hybrid GAL-CYC promoter.

23. The method of claim 17, wherein said molecule further comprises a signal sequence operably linked to said nucleotide sequence, said signal sequence coding an expression for a leader sequence directing the secretion of the polypeptide.

24. The method of claim 23, wherein the signal sequence is derived from the signal sequence of the killer toxin gene of Kluyveromyces lactis.

25. The method of claim 21, in which the host cell is of the species Saccharomyces cerevisiae.

26. The method of claim 17, wherein the host cell is a mammalian cell.

27. The method of claim 26, wherein the host cell is transformed with a retroviral expression vector.

28. The method of claim 26, wherein the vector is derived from the Moloney murine leukaemia virus.

29. The method of claim 28, wherein the vector further comprises the LTR enhancer of myeloproliferative sarcoma virus.

30. The method of claim 29, wherein said vector lacks the 3' untranslated region of native LIF mRNA.

31. The method of claim 26, wherein the host cells are hemopoietic cells.

32. The method of claim 17, wherein the host cells are E.coli cells.

33. The method of claim 32, wherein the vector is one which directs the expression of a glutathione S-transferase/LIF fusion protein.

34. The method of claim 33, wherein the vector is one which direct the expression of a glutathione S-transferase/thrombin cleavage site/LIF fusion protein.

35. A LIF polypeptide, obtainable by the method of claim 17.

36. A LIF polypeptide as defined in claim 1, which has an amino acid sequence including amino acid residues 179-187 of natural murine LIF as set forth in Figure 15.

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37. The polypeptide of claim 36, which has an N-terminal which lacks at least the first three amino acid residues of natural murine LIF.

38. Use of a LIF polypeptide having LIF activity as defined in any one of claims 1 to 5 and 35 to 37 for the preparation of a composition for hindering the proliferation of myeloid leukaemia cells.

39. Use of claimed in claim 38, for the preparation of a composition further comprising a polypeptide having G-CSF or GM-CSF activity.

40. A pharmaceutical composition comprising a LIF polypeptide as defined in any one of claims 1 to 5 and 35 to 37 in association with one or more pharmaceutically acceptable carriers and/or diluents.

41. The composition of claim 40, wherein the mature LIF polypeptide is fully homologous with the amino acid sequence set forth in Figure 29.

42. The composition of claim 40 or claim 41, further comprising at least one other biological regulator of blood cells.

43. The composition of claim 42, wherein said other regulator is a polypeptide having G-CSF or GM-CSF activity.

44. A diagnostic reagent or probe comprising at least one nucleic acid sequence corresponding to a sequence or subsequence of an LIF polypeptide encoding insert selected from the group consisting of the LIF-encoding inserts of clones pLIF7,2b, pLIFNK1, pLIFNK3, and pHGLIFBamI as defined in Figures 10, 11, 15 or 25, or to a sequence or subsequence of the corresponding mRNAs.

Claims for the following Contracting State : ES

1. A method for preparing a polypeptide having leukaemia inhibitory factor (LIF) activity, wherein said polypeptide is capable of suppressing the proliferation of M1 myeloid leukaemic cells, comprises the amino acid sequence set forth in Figures 15, 26 or 29 either completely or in part and is capable of competing with a polypeptide of said sequence for binding to specific cellular receptors on M1 cells or murine or human macrophages, said method comprising either, expressing in a host cell a corresponding nucleotide sequence encoding said polypeptide or chemical peptide synthesis.

2. A method for preparing a polypeptide having leukaemia inhibitory factor (LIF) activity, wherein said polypeptide is capable of suppressing the proliferation of M1 myeloid leukaemic cells, is encoded by a nucleotide sequence which is capable of hybridising under conditions of 6xSSC, 65 °C or conditions of higher stringency, to a sequence of a LIF encoding insert selected from the LIF-encoding inserts of clones pLIF7.2b, pLIFNK1, pLIFNK3, and pHGLIFBamI as defined in Figures 10, 11, 15 or 25 and is capable of competing with a polypeptide of the amino acid sequence set forth in Figures 15, 26 or 29 for binding to specific cellular receptors on M1 cells or murine or human macrophages, said method comprising either, expressing in a host cell a corresponding nucleotide sequence encoding said polypeptide or chemical peptide synthesis.

3. A method for preparing a polypeptide having leukaemia inhibitory factor (LIF) activity wherein said polypeptide:
   (a) is capable of suppressing the proliferation of M1 myeloid leukaemic cells;
   (b) comprises the amino acid sequence set forth in Figures 15, 26 or 29 either completely or in part;
   (c) is capable of competing with a polypeptide of said sequence for binding to specific cellular receptors on M1 cells or murine or human macrophages; and
   (d) is encoded by a nucleotide sequence which is capable of hybridising under conditions of 6xSSC, 65 °C or conditions of higher stringency, to a sequence of a LIF encoding insert selected from the LIF-encoding inserts of clones pLIF7.2b, pLIFNK1, pLIFNK3, and pHGLIFBamI as defined in Figures 10, 11, 15 or 25, said method comprising either, expressing in a host cell a corresponding nucleotide sequence encoding said polypeptide or chemical peptide synthesis.

* C L M S *

4. A method for preparing a polypeptide having leukaemia inhibitory factor (LIF) activity, wherein said polypeptide is capable of suppressing the proliferation of M1 myeloid leukaemic cells, comprises the amino acid sequence set forth in Figures 15, 26 or 29 either completely or in part or a sequence which has at least 78% amino acid homology therewith, and is capable of competing with a polypeptide of said sequence for binding to specific cellular receptors on M1 cells or murine or human macrophages, said method comprising either, expressing in a host cell a corresponding nucleotide sequence encoding said polypeptide or chemical peptide synthesis.

5. A method for preparing a polypeptide leukaemia inhibitory factor (LIF) activity which is capable of suppressing the proliferation of M1 myeloid leukaemic cells and which comprises the amino acid sequence set forth in Figures 15, 26 or 29, said method comprising either, expressing in a host cell a corresponding nucleotide sequence encoding said polypeptide or chemical peptide synthesis.

6. A method of purifying a glycosylated or non-glycosylated LIF polypeptide as defined in claim 1, which comprises (a) chromatographing LIF in crude form on an anion exchange column, and eluting LIF by an increasing salt gradient, (b) chromatographing the product of step (a) on a lectin affinity column, said lectin having an affinity for mannose, and eluting LIF with a mannose derivative, (c) chromatographing the product of step (b) on a cation exchange column and eluting LIF with an increasing salt gradient, (d) chromatographing the product of step (c) under HPLC conditions on a reverse phase column and eluting LIF with an increasing gradient of acetonitrile.

7. The method of claim 6, wherein the LIF in crude form comprises a Krebs II ascites tumour cell conditioned medium, and murine LIF is purified from said medium.

8. The method of claim 6, wherein the LIF in crude form comprises human bladder carcinoma cell line 5637 (ATCC deposit No. HTB9) conditioned medium, and human LIF is purified from said medium.

9. A method for preparing a recombinant DNA molecule comprising a nucleotide sequence which codes for a LIF polypeptide as defined in any one of claims 1 to 5, said method comprising the step of inserting said nucleotide sequence into vector DNA.

10. A method for preparing a recombinant DNA molecule comprising a nucleotide sequence as set forth in Figures 10, 15 (bases 1-630), 25 or 29 (bases 1-543) completely or in part, which encodes a polypeptide capable of suppressing the proliferation of M1 myeloid leukaemic cells and competing with a polypeptide having an amino acid sequence set forth in Figures 15, 26 or 29 for binding to specific cellular receptors on M1 cells or murine or human macrophages, said method comprising the step of inserting said nucleotide sequence into vector DNA.

11. A method for preparing a recombinant DNA molecule comprising a nucleotide sequence which codes on expression in a suitable host cell for a LIF polypeptide as defined in claim 1, wherein said nucleotide sequence hybridizes under conditions of 6xSSC, 65 °C or conditions of higher stringency, to a sequence of a LIF encoding insert selected from the LIF-encoding inserts of clones pLIF7.2b, pLIFNK1, pLIFNK3, and pHGLIFBamI as defined in Figures 10, 11, 15 or 25, said method comprising the step of inserting said nucleotide sequence into vector DNA.

12. A method for preparing a recombinant DNA molecule comprising a nucleotide sequence which codes on expression in a suitable host cell for a LIF polypeptide as defined in claim 1, wherein the nucleotide sequence codes on expression in a eukaryotic host for a polypeptide which comprises a mature polypeptide which is at least 78% homologous with a mature polypeptide amino acid sequence selected from those set forth in Figures 15, 26 and 29, said method comprising the step of inserting said nucleotide sequence into vector DNA.

13. The method of claim 12, wherein the mature polypeptide has full homology with the amino acid sequence set forth in Figure 29.

14. The method of any one of claims 9 to 13, further comprising inserting an origin of replication, whereby said molecule is rendered suitable for use as a cloning vector.

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15. The method of claim 14, further comprising inserting a promoter sequence operably linked to said nucleotide sequence whereby said molecule is rendered suitable for use as an expression vector.

16. A method of preparing a host cell capable of expressing a LIF polypeptide as defined in any of the preceding claims, comprising introducing into said host cell a recombinant DNA molecule as defined in claim 15.

17. A method of producing a LIF polypeptide as defined in claim 1 which comprises: (a) providing host cells prepared according to claim 16, (b) cultivating said cells under conditions conducive to expression of said polypeptide; and (c) recovering said polypeptide.

18. The method of claim 17, wherein the polypeptide comprises a mature polypeptide which is at least 78% homologous with an amino acid sequence selected from those set forth in Figures 15, 26 and 29.

19. The method of claim 17, wherein the mature polypeptide has full homology with the amino acid sequence set forth in Figure 29.

20. The method of claim 17, wherein the polypeptide is glycosylated by the host cell.

21. The method of claim 17, wherein the host cell is a yeast cell.

22. The method of claim 17, wherein the promoter is a galactose inducible hybrid GAL-CYC promoter.

23. The method of claim 17, wherein said molecule further comprises a signal sequence operably linked to said nucleotide sequence, said signal sequence coding an expression for a leader sequence directing the secretion of the polypeptide.

24. The method of claim 23, wherein the signal sequence is derived from the signal sequence of the killer toxin gene of Kluyveromyces lactis.

25. The method of claim 21, in which the host cell is of the species Saccharomyces cerevisiae.

26. The method of claim 17, wherein the host cell is a mammalian cell.

27. The method of claim 26, wherein the host cell is transformed with a retroviral expression vector.

28. The method of claim 26, wherein the vector is derived from the Moloney murine leukaemia virus.

29. The method of claim 28, wherein the vector further comprises the LTR enhancer of myeloproliferative sarcoma virus.

30. The method of claim 29, wherein said vector lacks the 3' untranslated region of native LIF mRNA.

31. The method of claim 26, wherein the host cells are hemopoietic cells.

32. The method of claim 17, wherein the host cells are E.coli cells.

33. The method of claim 32, wherein the vector is one which directs the expression of a glutathione S-transferase/LIF fusion protein.

34. The method of claim 33, wherein the vector is one which direct the expression of a glutathione S-transferase/thrombin cleavage site/LIF fusion protein.

35. A method of preparing a LIF polypeptide according to claim 1, wherein said polypeptide has an amino acid sequence including amino acid residues 179-187 of natural murine LIF as set forth in Figure 15.

36. A method according to claim 35, wherein said polypeptide has an N-terminal which lacks at least the first three amino acid residues of natural murine LIF.

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37. Use of a LIF polypeptide having LIF activity as defined in any one of claims 1 to 5 and 35 to 37 for the preparation of a composition for hindering the proliferation of myeloid leukaemia cells.

38. Use as claimed in claim 37, for the preparation of a composition further comprising a polypeptide having G-CSF or GM-CSF activity.

39. A method of preparing a pharmaceutical composition comprising a LIF polypeptide as defined in any one of claims 1 to 5 and 35 to 37, comprising admixing said polypeptide with one or more pharmaceutically acceptable carriers and/or diluents.

40. The method of claim 39, wherein the mature LIF polypeptide is fully homologous with the amino acid sequence set forth in Figure 29.

41. The method of claim 39 or claim 40, further comprising admixing at least one other biological regulator of blood cells.

42. The method of claim 41, wherein said other regulator is a polypeptide having G-CSF or GM-CSF activity.

Claims for the following Contracting State : GR

1. A method for preparing a polypeptide having leukaemia inhibitory factor (LIF) activity, wherein said polypeptide is capable of suppressing the proliferation of M1 myeloid leukaemic cells, comprises the amino acid sequence set forth in Figures 15, 26 or 29 either completely or in part and is capable of competing with a polypeptide of said sequence for binding to specific cellular receptors on M1 cells or murine or human macrophages, said method comprising either, expressing in a host cell a corresponding nucleotide sequence encoding said polypeptide or chemical peptide synthesis.

2. A method for preparing a polypeptide having leukaemia inhibitory factor (LIF) activity, wherein said polypeptide is capable of suppressing the proliferation of M1 myeloid leukaemic cells, is encoded by a nucleotide sequence which is capable of hybridising under conditions of 6xSSC, 65 °C or conditions of higher stringency, to a sequence of a LIF encoding insert selected from the LIF-encoding inserts of clones pLIF7.2b, pLIFNK1, pLIFNK3, and pHGLIFBamI as defined in Figures 10, 11, 15 or 25 and is capable of competing with a polypeptide of the amino acid sequence set forth in Figures 15, 26 or 29 for binding to specific cellular receptors on M1 cells or murine or human macrophages, said method comprising either, expressing in a host cell a corresponding nucleotide sequence encoding said polypeptide or chemical peptide synthesis.

3. A method for preparing a polypeptide having leukaemia inhibitory factor (LIF) activity wherein said polypeptide:
   (a) is capable of suppressing the proliferation of M1 myeloid leukaemic cells;
   (b) comprises the amino acid sequence set forth in Figures 15, 26 or 29 either completely or in part;
   (c) is capable of competing with a polypeptide of said sequence for binding to specific cellular receptors on M1 cells or murine or human macrophages; and
   (d) is encoded by a nucleotide sequence which is capable of hybridising under conditions of 6xSSC, 65 °C or conditions of higher stringency, to a sequence of a LIF encoding insert selected from the LIF-encoding inserts of clones pLIF7.2b, pLIFNK1, pLIFNK3, and pHGLIFBamI as defined in Figures 10, 11, 15 or 25, said method comprising either, expressing in a host cell a corresponding nucleotide sequence encoding said polypeptide or chemical peptide synthesis.

4. A method for preparing a polypeptide having leukaemia inhibitory factor (LIF) activity, wherein said polypeptide is capable of suppressing the proliferation of M1 myeloid leukaemic cells, comprises the amino acid sequence set forth in Figures 15, 26 or 29 either completely or in part or a sequence which has at least 78% amino acid homology therewith, and is capable of competing with a polypeptide of said sequence for binding to specific cellular receptors on M1 cells or murine or human macrophages, said method comprising either, expressing in a host cell a corresponding nucleotide sequence encoding said polypeptide or chemical peptide synthesis.

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5. A method for preparing a polypeptide leukaemia inhibitory factor (LIF) activity which is capable of suppressing the proliferation of M1 myeloid leukaemic cells and which comprises the amino acid sequence set forth in Figures 15, 26 or 29, said method comprising either, expressing in a host cell a corresponding nucleotide sequence encoding said polypeptide or chemical peptide synthesis.

6. A method of purifying a glycosylated or non-glycosylated LIF polypeptide as defined in claim 1, which comprises (a) chromatographing LIF in crude form on an anion exchange column, and eluting LIF by an increasing salt gradient, (b) chromatographing the product of step (a) on a lectin affinity column, said lectin having an affinity for mannose, and eluting LIF with a mannose derivative, (c) chromatographing the product of step (b) on a cation exchange column and eluting LIF with an increasing salt gradient, (d) chromatographing the product of step (c) under HPLC conditions on a reverse phase column and eluting LIF with an increasing gradient of acetonitrile.

7. The method of claim 6, wherein the LIF in crude form comprises a Krebs II ascites tumour cell conditioned medium, and murine LIF is purified from said medium.

The method of claim 6, wherein the LIF in crude form comprises human bladder carcinoma cell line 5637 (ATCC deposit No. HTB9) conditioned medium, and human LIF is purified from said medium.

9. A recombinant DNA molecule comprising a nucleotide sequence which codes for a LIF polypeptide as defined in any one of claims 1 to 5.

10. A recombinant DNA molecule comprising a nucleotide sequence as set forth in Figures 10, 15 (bases 1-630), 25 or 29 (bases 1-543) completely or in part, which encodes a polypeptide capable of suppressing the proliferation of M1 myeloid leukaemic cells and competing with a polypeptide having an amino acid sequence set forth in Figures 15, 26 or 29 for binding to specific cellular receptors on M1 cells or murine or human macrophages.

11. A recombinant DNA molecule comprising a nucleotide sequence which codes on expression in a suitable host cell for a LIF polypeptide as defined in claim 1, wherein said nucleotide sequence hybridizes under conditions of 6xSSC, 65 °C or conditions of higher stringency, to a sequence of a LIF encoding insert selected from the LIF-encoding inserts of clones pLIF7.2b, pLIFNK1, pLIFNK3, and pHGLIFBamI as defined in Figures 10, 11, 15 or 25.

12. A recombinant DNA molecule comprising a nucleotide sequence which codes on expression in a suitable host cell for a LIF polypeptide as defined in claim 1, wherein the nucleotide sequence codes on expression in a eukaryotic host for a polypeptide which comprises a mature polypeptide which is at least 78% homologous with a mature polypeptide amino acid sequence selected from those set forth in Figures 15, 26 and 29.

13. The molecule of claim 12, wherein the mature polypeptide has full homology with the amino acid sequence set forth in Figure 29.

14. The molecule of any one of claims 9 to 13, further comprising an origin of replication, whereby said molecule is rendered suitable for use as a cloning vector.

15. The molecule as claim 14, further comprising a promoter sequence operably linked to said nucleotide sequence whereby said molecule is rendered suitable for use of an expression vector.

16. A host cell containing a recombinant DNA molecule according to claim 15.

17. A method of producing a LIF polypeptide as defined in claim 1 which comprises: (a) providing host cells containing a molecule according to claim 14, (b) cultivating said cells under conditions conducive to expression of said polypeptide; and (c) recovering said polypeptide.

18. The method of claim 17, wherein the polypeptide comprises a mature polypeptide which is at least 78% homologous with an amino acid sequence selected from those set forth in Figures 15, 26 and 29.

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19. The method of claim 17, wherein the mature polypeptide has full homology with the amino acid sequence set forth in Figure 29.

20. The method of claim 17, wherein the polypeptide is glycosylated by the host cell.

21. The method of claim 17, wherein the host cell is a yeast cell.

22. The method of claim 17, wherein the promoter is a galactose inducible hybrid GAL-CYC promoter.

23. The method of claim 17, wherein said molecule further comprises a signal sequence operably linked to said nucleotide sequence, said signal sequence coding an expression for a leader sequence directing the secretion of the polypeptide.

24. The method of claim 23, wherein the signal sequence is derived from the signal sequence of the killer toxin gene of Kluyveromyces lactis.

25. The method of claim 21, in which the host cell is of the species Saccharomyces cerevisiae.

26. The method of claim 17, wherein the host cell is a mammalian cell.

27. The method of claim 26, wherein the host cell is transformed with a retroviral expression vector.

28. The method of claim 26, wherein the vector is derived from the Moloney murine leukaemia virus.

29. The method of claim 28, wherein the vector further comprises the LTR enhancer of myeloproliferative sarcoma virus.

30. The method of claim 29, wherein said vector lacks the 3' untranslated region of native LIF mRNA.

31. The method of claim 26, wherein the host cells are hemopoietic cells.

32. The method of claim 17, wherein the host cells are E.coli cells.

33. The method of claim 32, wherein the vector is one which directs the expression of a glutathione S-transferase/LIF fusion protein.

34. The method of claim 33, wherein the vector is one which direct the expression of a glutathione S-transferase/thrombin cleavage site/LIF fusion protein.

35. A method of preparing a LIF polypeptide according to claim 1, wherein said polypeptide has an amino acid sequence including amino acid residues 179-187 of natural murine LIF as set forth in Figure 15.

36. A method according to claim 35, wherein said polypeptide has an N-terminal which lacks at least the first three amino acid residues of natural murine LIF.

37. Use of a LIF polypeptide having LIF activity as defined in any one of claims 1 to 5 and 35 to 37 for the preparation of a composition for hindering the proliferation of myeloid leukaemia cells.

38. Use as claimed in claim 37, for the preparation of a composition further comprising a polypeptide having G-CSF or GM-CSF activity.

39. A method of preparing a pharmaceutical composition comprising a LIF polypeptide as defined in any one of claims 1 to 5 and 35 to 37, comprising admixing said polypeptide with one or more pharmaceutically acceptable carriers and/or diluents.

40. The method of claim 39, wherein the mature LIF polypeptide is fully homologous with the amino acid sequence set forth in Figure 29.

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41. The method of claim 39 or claim 40, further comprising admixing at least one other biological regulator of blood cells.

42. The method of claim 41, wherein said other regulator is a polypeptide having G-CSF or GM-CSF activity.

Patentansprüche
Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, LI, DE, FR, GB, IT, LU, NL, SE

1. Polypeptid mit Leukämiehemmfaktor (LIF)-Aktivität, dadurch gekennzeichnet, daß es die Proliferierung von leukämischen, myeloischen M1-Zellen zu supprimieren vermag, daß es die in den Figuren 15, 26 oder 29 dargestellte Aminosäuresequenz entweder vollständig oder teilweise umfaßt, und daß es mit einem Polypeptid dieser Sequenz um die Bindung an spezifischen zellulären Rezeptoren auf M1 Zellen oder Mäuse- oder Humanmakrophagen zu kompetitieren vermag.

2. Polypeptid mit Leukämiehemmfaktor (LIF)-Aktivität, dadurch gekennzeichnet, daß es die Proliferierung von leukämischen, myeloischen M1-Zellen zu supprimieren vermag, daß es durch eine Nukleotidsequenz codiert ist, die unter Bedingungen von 6xSSC, 65 °C oder Bedingungen höherer Strenge mit einer Sequenz eines für LIF codierenden Inserts, ausgewählt aus den für LIF codierenden Inserten der Klone pLIF7.2b, pLIFNK1, pLIFNK3 und pHGLIFBamI gemäß den Definitionen in den Figuren 10, 11, 15 oder 25, zu hybridisieren vermag, und daß es mit einem Polypeptid der in den Figuren 15, 26 oder 29 dargestellten Aminosäuresequenz um die Bindung an spezifischen zellulären Rezeptoren auf M1 Zellen oder Mäuse- oder Humanmakrophagen zu kompetitieren vermag.

3. Polypeptid mit Leukämiehemmfaktor (LIF)-Aktivität, dadurch gekennzeichnet, daß
   (a) es die Proliferierung von leukämischen, myeloischen M1-Zellen zu supprimieren vermag,
   (b) es die in den Figuren 15, 26 oder 29 dargestellte Aminosäuresequenz entweder vollständig oder teilweise umfaßt,
   (c) es mit einem Polypeptid dieser Sequenz um die Bindung an spezifischen zellulären Rezeptoren auf M1 Zellen oder Mäuse- oder Humanmakrophagen zu kompetitieren vermag, und
   (d) es durch eine Nukleotidsequenz codiert ist, die unter Bedingungen von 6xSSC, 65 °C oder Bedingungen höherer Strenge an eine Sequenz eines für LIF codierenden Inserts, ausgewählt aus den für LIF codierenden Inserten der Klone pLIF7.2b, pLIFNK1, pLIFNK3 und pHGLIFBamI entsprechend den Definitionen in den Figuren 10, 11, 15 oder 25, zu hybridisieren vermag.

4. Polypeptid mit Leukämiehemmfaktor (LIF)-Aktivität, dadurch gekennzeichnet, daß es die Profilierung von leukämischen, myeloischen M1-Zellen zu supprimieren vermag, daß es die in den Figuren 15, 26 oder 29 dargestellte Aminosäuresequenz entweder vollständig oder teilweise oder eine Sequenz, die mindestens 78% Aminosäurehomologie damit aufweist, umfaßt, und dadurch, daß sie mit einem Polypeptid dieser Sequenz um die Bindung an spezifische zelluläre Rezeptoren auf M1 Zellen oder Mäuse- oder Humanmakrophagen zu kompetitieren vermag.

5. Polypeptid mit Leukämiehemmfaktor (LIF)-Aktivität, das die Profilierung von leukämischen, myeloischen M1-Zellen zu supprimieren vermag und die in den Figuren 15, 26 oder 29 dargestellte Aminosäuresequenz umfaßt.

6. Verfahren zur Reinigung eines glykosylierten oder nicht-glykosylierten LIF-Polypeptids nach Anspruch 1, umfassend
   (a) das Chromatographieren von LIF in Rohform auf einer Anionenaustauschersäule und das Eluieren von LIF mit Hilfe eines zunehmenden Salzgradienten,
   (b) das Chromatographieren des Produkts aus Stufe (a) auf einer Lectin-Affinitätssäule, wobei das Lectin eine Affinität für Mannose aufweist, und das Eluieren von LIF mit einem Mannosederivat,
   (c) das Chromatographieren des Produkts aus Stufe (b) auf einer Kationenaustauschersäule und das Eluieren von LIF mit einem steigenden Salzgradienten,
   (d) das Chromatographieren des Produkts von Stufe (c) unter HPLC-Bedingungen auf einer Säule mit reverser Phase und das Eluieren von LIF mit einem zunehmenden Acetonitrilgradienten.

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7. Verfahren nach Anspruch 6, wobei der LIF in Rohform ein mit Krebs II-Aszitestumorzellen konditioniertes Medium enthält und Mäuse-LIF aus diesem Medium gereinigt wird.

8. Verfahren nach Anspruch 6, wobei der LIF in Rohform ein mit einer menschlichen Blasenkarzinomzelllinie 5637 (ATCC Hinterlegungsnummer HTB9) konditioniertes Medium enthält und menschliches LIF aus diesem Medium gereinigt wird.

9. Rekombinantes DNA-Molekül mit einer Nukleotidsequenz, die für ein LIF-Polypeptid nach einem der Ansprüche 1 bis 5 codiert.

10. Rekombinantes DNA-Molekül mit einer in den Figuren 10, 15 (Basen 1-630), 25 oder 29 (Basen 1-543) vollständig oder teilweise dargestellten Nukleotidsequenz, das für ein Polypeptid mit der Fähigkeit zum Supprimieren der Profilierung von leukämischen, myeloischen M1-Zellen und zum Kompetitieren mit einem Polypeptid mit einer in den Figuren 15, 26 oder 29 dargestellten Aminosäuresequenz um die Bindung an spezifische zelluläre Rezeptoren auf M1 Zellen oder Mäuse- oder Humanmakrophagen codiert.

11. Rekombinantes DNA-Molekül mit einer Nukleotidsequenz, die bei Expression in einer geeigneten Wirtszelle für ein LIF-Polypeptid nach Anspruch 1 codiert, wobei die Nukleotidsequenz unter Bedingungen von 6xSSC, 65 °C oder Bedingungen höherer Strenge mit einer Sequenz eines für LIF codierenden Inserts, ausgewählt aus den für LIF codierenden Inserten der Klone pLIF7.2b, pLIFNK1, pLIFNK3 und pHGLIFBamI entsprechend den Definitionen in Figuren 10, 11, 15 oder 25, hybridisiert.

12. Rekombinantes DNA-Molekül mit einer Nukleotidsequenz, die bei Expression in einer geeigneten Wirtszelle für ein LIF-Polypeptid nach Anspruch 1 codiert, wobei die Nukleotidsequenz bei Expression in einem eukaryontischen Wirt für ein Polypeptid codiert, das ein reifes Polypeptid umfaßt, welches zumindest zu 78% mit einer Aminosäuresequenz eines reifen Polypeptids, ausgewählt aus den in den Figuren 15, 26 und 29 dargestellten, homolog ist.

13. Molekül nach Anspruch 12, wobei das reife Polypeptid eine vollständige Homologie mit der in Figur 29 dargestellten Aminosäuresequenz aufweist.

14. Molekül nach einem der Ansprüche 9 bis 13, des weiteren umfassend einen Replikationsursprung, wobei das Molekül in einen zur Verwendung als Klonierungsvektor geeigneten Zustand gebracht wurde.

15. Molekül nach Anspruch 14, des weiteren umfassend eine funktionell mit der Nukleotidsequenz verknüpfte ("operably linked") Promotorsequenz, wobei das Molekül in einen zur Verwendung eines Expressionsvektors geeigneten Zustand gebracht wurde.

16. Wirtszelle mit einem rekombinanten DNA-Molekül nach Anspruch 15.

17. Verfahren zur Herstellung eines LIF-Polypeptids nach Anspruch 1, umfassend:
(a) das Bereitstellen von Wirtszellen mit einem Molekül nach Anspruch 14,
(b) das Kultivieren dieser Zellen unter Bedingungen, die die Expression des Polypeptids fördern, und
(c) das Gewinnen des Polypeptids.

18. Verfahren nach Anspruch 17, wobei das Polypeptid ein reifes Polypeptid umfaßt, das zumindest zu 78% mit einer Aminosäuresequenz, ausgewählt unter den in den Figuren 15, 26 und 29 dargestellten, homolog ist, umfaßt.

19. Verfahren nach Anspruch 17, wobei das reife Polypeptid eine vollständige Homologie mit der in Figur 29 dargestellten Aminosäuresequenz aufweist.

20. Verfahren nach Anspruch 17, wobei das Polypeptid durch die Wirtszelle glykosyliert ist.

21. Verfahren nach Anspruch 17, wobei die Wirtszelle aus einer Hefezelle besteht.

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22. Verfahren nach Anspruch 17, wobei der Promotor aus einem galaktoseindusierbaren Hybrid-GAL-CYC-Promotor besteht.

23. Verfahren nach Anspruch 17, wobei das Molekül des weiteren eine funktionell mit der Nukleotidsequenz verknüpfte ("operably linked") Signalsequenz umfaßt, wobei die Signalsequenz für eine Expression für eine auf die Absonderung des Polypeptids gerichtete Leadersequenz codiert.

24. Verfahren nach Anspruch 23, wobei die Signalsequenz von der Signalsequenz des Killertoxingens Kluyveromyces lactis abgeleitet ist.

25. Verfahren nach Anspruch 21, bei dem die Wirtszelle aus einer der Spezies Saccharomyces cerevisiae besteht.

26. Verfahren nach Anspruch 17, wobei die Wirtszelle aus einer Säugetierzelle besteht.

27. Verfahren nach Anspruch 26, wobei die Wirtszelle mit einem retroviralen Expressionsvektor transformiert ist.

28. Verfahren nach Anspruch 26, wobei der Vektor von dem Moloney-Mäuseleukämievirus abgeleitet ist.

29. Verfahren nach Anspruch 28, wobei der Vektor des weiteren den LTR-Verstärker eines myeloproliferativen Sarkomvirus umfaßt.

30. Verfahren nach Anspruch 29, wobei dem Vektor der nichttranslatierte 3'-Bereich nativer LIF-mRNA fehlt.

31. Verfahren nach Anspruch 26, wobei die Wirtszellen aus blutbildenden Zellen bestehen.

32. Verfahren nach Anspruch 17, wobei die Wirtszellen aus E. coli-Zellen bestehen.

33. Verfahren nach Anspruch 32, wobei der Vektor aus einem besteht, der auf die Expression eines Glutathion-S-Transferase/LIF-Fusionsproteins gerichtet ist.

34. Verfahren nach Anspruch 33, wobei der Vektor aus einem besteht, der auf die Expression eines Glutathion-S-Transferase/Thrombin-Spaltungsstelle/LIF-Fusionsproteins gerichtet ist.

35. LIF-Polypeptid, erhältlich nach dem Verfahren von Anspruch 17.

36. LIF-Polypeptid nach Anspruch 1, das eine Aminosäuresequenz einschließlich den Aminosäureresten 179-187 von natürlichem Mäuse-LIF gemäß Figur 15 aufweist.

37. Polypeptid nach Anspruch 36, das ein N-Terminus aufweist, dem mindestens die ersten drei Aminosäurereste natürlichen Mäuse-LIFs fehlen.

38. Verwendung eines LIF-Polypeptids mit LIF-Aktivität nach einem der Ansprüche 1 bis 5 und 35 bis 37 zur Herstellung einer Zubereitung zur Verhinderung der Proliferierung von leukämischen myeloischen Zellen.

39. Verwendung nach Anspruch 38 zur Herstellung einer Zubereitung, des weiteren umfassend ein Polypeptid mit G-CSF- oder GM-CSF-Aktivität.

40. Pharmazeutische Zubereitung mit einem LIF-Polypeptid nach einem der Ansprüche 1 bis 5 und 35 bis 37 in Verbindung mit einem oder mehreren pharmazeutisch akzeptablen Trägern und/oder Verdünnungsmitteln.

41. Zubereitung nach Anspruch 40, wobei das reife LIF-Polypeptid vollständig mit der in Fig. 29 dargestellten Aminosäuresequenz homolog ist.

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42. Zusammensetzung nach Anspruch 40 oder 41, des weiteren umfassend mindestens einen weiteren biologischen Regulator von Blutzellen.

43. Zusammensetzung nach Anspruch 42, wobei der weitere Regulator aus einem Polypeptid mit G-CSF- oder GM-CSF-Aktivität besteht.

44. Diagnostisches Mittel oder Sonde mit mindestens einer Nukleinsäuresequenz, die einer Sequenz oder Untersequenz eines für LIF-Polypeptid codierenden Inserts, ausgewählt aus der Gruppe der für LIF codierenden Inserte von Klone pLIF7.2b, pLIFNK1, pLIFNK3 und pHGLIFBamI entsprechend den Definitionen in den Figuren 10, 11, 15 oder 25, oder einer Sequenz oder Untersequenz der entsprechenden mRNAen entspricht.

Patentansprüche für folgenden Vertragsstaat : ES

1. Verfahren zur Herstellung eines Polypeptids mit Leukämiehemmfaktor (LIF)-Aktivität, wobei das Polypeptid die Profilierung von leukämischen, myeloischen M1-Zellen zu supprimieren vermag, daß es die in den Figuren 15, 26 oder 29 dargestellte Aminosäuresequenz entweder vollständig oder teilweise umfaßt, und daß es mit einem Polypeptid dieser Sequenz um eine Bindung an spezifischen zellulären Rezeptoren aud M1 Zellen oder Mäuse- oder Humanmakrophagen zu kompetitieren vermag, wobei das Verfahren entweder ein Exprimieren einer entsprechenden Nukleotidsequenz, die für das Polypeptid codiert, in einer Wirtszelle oder eine chemische Peptidsynthese umfaßt.

2. Verfahren zur Herstellung eines Polypeptids mit Leukämiehemmfaktor (LIF)-Aktivität, wobei das Polypeptid die Profilierung von leukämischen, myeloischen M1-Zellen zu supprimieren vermag, daß er durch eine Nukleotidsequenz codiert ist, die unter Bedingungen von 6xSSC, 65 °C oder Bedingungen höherer Strenge mit einer Sequenz eines für LIF codierenden Inserts, ausgewählt aus den für LIF codierenden Inserten der Klonen pLIF7.2b, pLIFNK1, pLIFNK3 und pHGLIFBamI entsprechend den Definitionen in den Figuren 10, 11, 15 oder 25, zu hybridisieren vermag, und daß es mit einem Polypeptid der in den Figuren 15, 26 oder 29 dargestellten Aminosäuresequenz um eine Bindung an spezifischen zellulären Rezeptoren auf M1 Zellen oder Mäuse- oder Humanmakrophagen zu kompetitieren vermag, wobei das Verfahren entweder ein Exprimieren einer entsprechenden Nukleotidsequenz, die für das Polypeptid codiert, in einer Wirtszelle oder eine chemische Peptidsynthse umfaßt.

3. Verfahren zur Herstellung eines Polypeptids mit Leukämiehemmfaktor (LIF)-Aktivität, wobei das Polypeptid
   (a) die Profilierung von M1-Zellen zu supprimieren vermag,
   (b) die in den Figuren 15, 26 oder 29 dargestellte Aminosäuresequenz entweder vollständig oder teilweise umfaßt,
   (c) mit einem Polypeptid der Sequenz um eine Bindung an spezifischen zellulären Rezeptoren auf M1-Zellen oder Mäuse- oder Humanmakrophagen zu kompetitieren vermag, und
   (d) durch eine Nukleotidsequenz codiert ist, die unter Bedingungen von 6xSSC, 65 °C oder Bedingungen höherer Strenge mit einer Sequenz eines für LIF codierenden Inserts, ausgewählt aus den für LIF codierenden Inserten der Klone pLIF7.2b, pLIFNK1, pLIFNK3 und pHGLIFBamI entsprechend den Definitionen in den Figuren 10, 11, 15 oder 25, zu hybridisieren vermag, wobei das Verfahren entweder ein Exprimieren einer entsprechenden Nukleotidsequenz, die für das Polypeptid codiert, in einer Wirtszelle oder eine chemische Peptidsynthese umfaßt.

4. Verfahren zur Herstellung eines Polypeptids mit Leukämiehemmfaktor (LIF)-Aktivität, wobei das Polypeptid die Poliferierung von leukämischen, myeloischen M1-Zellen zu supprimieren vermag, die in den Figuren 15, 26 oder 29 dargestellte Aminosäuresequenz entweder vollständig oder teilweise oder eine Sequenz, die eine zu mindestens 78% betragende Aminosäurehomologie damit aufweist, umfaßt, und mit einem Polypeptid der Sequenz um die Bindung an spezifischen zelluläre Rezeptoren auf M1-Zellen oder Mäuse- oder Humanmakrophagen zu kompetitieren vermag, wobei das Verfahren entweder ein Exprimieren einer entsprechenden Nukleotidsequenz, die für das Polypeptid codiert, in einer Wirtszelle oder eine chemische Peptidsynthese umfaßt.

5. Verfahren zur Herstellung eines Polypeptids mit Leukämiehemmfaktor (LIF)-Aktivität, das die Proliferierung von leukämischen, myeloischen M1-Zellen zu supprimieren vermag und die in den Figuren 15, 26

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oder 29 dargestellte Aminosäuresequenz umfaßt, wobei das Verfahren entweder ein Exprimieren einer entsprechenden Nukleotidsequenz, die für das Polypeptid codiert, in einer Wirtszelle oder eine chemische Peptidsynthese umfaßt.

6. Verfahren zur Reinigung eines glykosylierten oder nicht-glykosylierten LIF-Polypeptids nach Anspruch 1, umfassend
(a) das Chromatographieren von LIF in Rohform auf einer Anionenaustauschersäule und das Eluieren von LIF mit Hilfe eines zunehmenden Salzgradienten,
(b) das Chromatographieren des Produkts aus Stufe (a) auf einer Lectin-Affinitätssäule, wobei das Lectin eine Affinität für Mannose aufweist, und das Eluieren von LIF mit einem Mannosederivat,
(c) das Chromatographieren des Produkts aus Stufe (b) auf einer Kationenaustauschersäule und das Eluieren von LIF mit einem zunehmenden Salzgradienten,
(d) das Chromatographieren des Produkts aus Stufe (c) unter HPLC-Bedingungen auf einer Säule mit reverser Phase und das Eluieren von LIF mit einem zunehmenden Acetonitrilgradienten.

7. Verfahren nach Anspruch 6, wobei der LIF in Rohform ein mit Krebs II-Aszitestumorzellen konditioniertes Medium enthält und Mäuse-LIF aus diesem Medium gereinigt wird.

8. Verfahren nach Anspruch 6, wobei der LIF in Rohform ein mit einer menschlichen Blasenkarzinomzellinie 5637 (ATCC Hinterlegungsnummer HTB9) konditioniertes Medium enthält und das menschliche LIF aus diesem Medium gereinigt wird.

9. Verfahren zur Herstellung eines rekombinanten DNA-Moleküls mit einer Nukleotidsequenz, die für ein LIF-Polypeptid nach einem der Ansprüche 1 bis 5 codiert, wobei das Verfahren den Schritt eines Insertierens der Nukleotidsequenz in eine Vektor-DNA umfaßt.

10. Verfahren zur Herstellung eines eine in den Figuren 10, 15 (Basen 1-630), 25 oder 29 (Basen 1-543) dargestellte Nukleotidsequenz vollständig oder teilweise umfassenden rekombinanten DNA-Moleküls, das für ein Polypeptid mit der Fähigkeit zur Supprimierung der Proliferierung von leukämischen, myeloischen M1-Zellen und zur Kompetitierung mit einem Polypeptid mit einer in den Figuren 15, 26 oder 29 dargestellten Aminosäuresequenz um eine Bindung an spezifischen zellulären Rezeptoren auf M1-Zellen oder Mäuse- oder Humanmakrophagen codiert, wobei das Verfahren die Stufe eines Insertierens der Nukleotidsequenz in eine Vektor-DNA umfaßt.

11. Verfahren zur Herstellung eines rekombinanten DNA-Moleküls mit einer Nukleotidsequenz, die bei Expression in einer geeigneten Wirtszelle für ein LIF-Polypeptid nach Anspruch 1 codiert, wobei die Nukleotidsequenz unter Bedingungen von 6xSSC, 65 °C oder Bedingungen höherer Strenge mit einer Sequenz eines für LIF codierenden Inserts, ausgewählt aus den für LIF codierenden Inserten der Klone pLIF7.2b, pLIFNK1, pLIFNK3 und pHGLIFBamI entsprechend den Definitionen in den Figuren 10, 11, 15 oder 25, hybridisiert, wobei das Verfahren die Stufe eines Insertierens der Nukleotidsequenz in Vektor-DNA umfaßt.

12. Verfahren zur Herstellung eines rekombinanten DNA-Moleküls mit einer Nukleotidsequenz, die bei Expression in einer geeigneten Wirtszelle für ein LIF-Polypeptid nach Anspruch 1 codiert, wobei die Nukleotidsequenz bei Expression in einem eukaryontischen Wirt für ein Polypeptid codiert, das ein reifes Polypeptid umfaßt, welches zu mindestens zu 78% mit einer Aminosäuresequenz eines reifen Polypeptids, ausgewählt aus den in den Figuren 15, 26 und 29 dargestellten, homolog ist, wobei das Verfahren die Stufe eines Insertierens der Nukleotidsequenz in Vektor-DNA umfaßt.

13. Verfahren nach Anspruch 12, wobei das reife Polypeptid eine vollständige Homologie mit der in Figur 29 dargestellten Aminosäuresequenz aufweist.

14. Verfahren nach einem der Ansprüche 9 bis 13, des weiteren umfassend ein Insertieren eines Replikationsursprungs, wobei das Molekül in eine zur Verwendung als Klonierungsvektor geeignete Form gebracht wurde.

15. Verfahren nach Anspruch 14, des weiteren umfassend ein Insertieren einer funktionell mit der Nukleotidsequenz verknüpften ("operably linked") Promotorsequenz, wobei das Molekül in eine zur Verwen-

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dung eines Expressionsvektors geeignete Form gebracht wurde.

16. Verfahren zur Herstellung einer Wirtszelle mit der Fähigkeit zur Expression eines LIF-Polypeptids nach einem der vorhergehenden Ansprüche, umfassend ein Einführen eines rekombinanten DNA-Moleküls nach Anspruch 15 in die Wirtszelle.

17. Verfahren zur Herstellung eines LIF-Polypeptids nach Anspruch 1, umfassend:
(a) das Bereitstellen von gemäß Anspruch 16 hergestellten Wirtszellen,
(b) das Kultivieren der Zellen unter Bedingungen, die einer Expression des Polypeptids förderlich sind und
(c) das Gewinnen des Polypeptids.

18. Verfahren nach Anspruch 17, wobei das Polypeptid ein reifes Polypeptid umfaßt, das zu mindestens zu 78% mit einer Aminosäuresequenz, ausgewählt aus den in den Figuren 15, 26 und 29 dargestellten, homolog ist.

19. Verfahren nach Anspruch 17, wobei das reife Polypeptid eine vollständige Homologie mit der in Figur 29 dargestellten Aminosäuresequenz aufweist.

20. Verfahren nach Anspruch 17, wobei das Polypeptid durch die Wirtszelle glykosyliert ist.

21. Verfahren nach Anspruch 17, wobei die Wirtszelle aus einer Hefezelle besteht.

22. Verfahren nach Anspruch 17, wobei der Promotor aus einem galaktoseinduzierbaren Hybrid-GAL-CYC-Promotor besteht.

23. Verfahren nach Anspruch 17, wobei das Molekül des weiteren eine funktionell mit der Nukleotidsequenz verknüpfte ("operably linked") Signalsequenz umfaßt, wobei die Signalsequenz für eine Expression für eine auf die Absonderung des Polypeptids gerichtete Leadersequenz codiert.

24. Verfahren nach Anspruch 23, wobei die Signalsequenz von der Signalsequenz des Killertoxingens Kluyveromyces lactis abgeleitet ist.

25. Verfahren nach Anspruch 21, bei dem die Wirtszelle aus einer der Spezies Saccharomyces cerevisiae besteht.

26. Verfahren nach Anspruch 17, wobei die Wirtszelle aus einer Säugetierzelle besteht.

27. Verfahren nach Anspruch 26, wobei die Wirtszelle mit einem retroviralen Expressionsvektor transformiert ist.

28. Verfahren nach Anspruch 26, wobei der Vektor von dem Moloney-Mäuseleukämievirus abgeleitet ist.

29. Verfahren nach Anspruch 28, wobei der Vektor des weiteren den LTR-Verstärker eines myeloproliferativen Sarkomvirus umfaßt.

30. Verfahren nach Anspruch 29, wobei dem Vektor der nichttranslatierte 3'-Bereich natürlicher LIF-mRNA fehlt.

31. Verfahren nach Anspruch 26, wobei die Wirtszellen aus blutbildenden Zellen bestehen.

32. Verfahren nach Anspruch 17, wobei die Wirtszellen E. coli-Zellen sind.

33. Verfahren nach Anspruch 32, wobei der Vektor aus einem besteht, der auf die Expression eines Glutathion-S-Transferase/LIF-Fusionsproteins gerichtet ist.

34. Verfahren nach Anspruch 33, wobei der Vektor aus einem besteht, der auf die Expression eines Glutathion-S-Transferase/Thrombin-Spaltungsstelle/LIF-Fusionsproteins gerichtet ist.

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35. Verfahren zur Herstellung eines LIF-Polypeptids nach Anspruch 1, wobei das Polypeptid eine Aminosäuresequenz einschließlich den Aminosäureresten 179-187 von natürlichem Mäuse-LIF gemäß Figur 15 aufweist.

36. Verfahren nach Anspruch 35, wobei das Polypeptid einen N-Terminus aufweist, dem mindestens die ersten drei Aminosäurereste natürlicher Mäuse-LIF fehlen.

37. Verwendung eines LIF-Polypeptids mit LIF-Aktivität nach einem der Ansprüche 1 bis 5 und 35 bis 37 zur Herstellung einer Zubereitung zur Hemmung der Proliferierung von leukämischen myeloischen Zellen.

38. Verwendung nach Anspruch 37 zur Herstellung einer Zubereitung, des weiteren umfassend ein Polypeptid mit G-CSF- oder GM-CSF-Aktivität.

39. Verfahren zur Herstellung einer pharmazeutischen Zubereitung, umfassend ein LIF-Polypeptid nach einem der Ansprüche 1 bis 5 und 35 bis 37, umfassend das Vermischen des Polypeptids mit einem oder mehreren pharmazeutisch akzeptablen Trägern und/oder Verdünnungsmitteln.

40. Verfahren nach Anspruch 39, wobei das reife LIF-Polypeptid vollständig mit der in Fig. 29 dargestellten Aminosäuresequenz homolog ist.

41. Verfahren nach Anspruch 39 oder Anspruch 40, des weiteren umfassend das Vermischen mit mindestens einem weiteren biologischen Regulator von Blutzellen.

42. Verfahren nach Anspruch 41, wobei der weitere Regulator aus einem Polypeptid mit G-CSF- oder GM-CSF-Aktivität besteht.

Patentansprüche für folgenden Vertragsstaat : GR

1. Verfahren zur Herstellung eines Polypeptids mit Leukämiehemmfaktor (LIF)-Aktivität, wobei das Polypeptid die Profilierung von leukämischen, myeloischen M1-Zellen zu supprimieren vermag, daß es die in den Figuren 15, 26 oder 29 dargestellte Aminosäuresequenz entweder vollständig oder teilweise umfaßt, und daß es mit einem Polypeptid dieser Sequenz um eine Bindung an spezifischen zellulären Rezeptoren auf M1-Zellen oder Mäuse- oder Humanmakrophagen zu kompetitieren vermag, wobei das Verfahren entweder ein Exprimieren einer entsprechenden Nukleotidsequenz, die für das Polypeptid codiert, in einer Wirtszelle oder eine chemische Peptidsynthese umfaßt.

2. Verfahren zur Herstellung eines Polypeptids mit Leukämiehemmfaktor (LIF)-Aktivität, wobei das Polypeptid die Proliferierung von leukämischen, myeloischen M1-Zellen zu supprimieren vermag, daß es durch eine Nukleotidsequenz codiert ist, die unter Bedingungen von 6xSSC, 65 °C oder Bedingungen höherer Strenge mit einer Sequenz eines für LIF codierenden Inserts, ausgewählt aus den für LIF codierenden Inserten der Klonen pLIF7.2b, pLIFNK1, pLIFNK3 und pHGLIFBamI entsprechend den Definitionen in den Figuren 10, 11, 15 oder 25, zu hybridisieren vermag, und daß es mit einem Polypeptid der in den Figuren 15, 26 oder 29 dargestellten Aminosäuresequenz um eine Bindung an spezifischen zellulären Rezeptoren auf M1-Zellen oder Mäuse- oder Humanmakrophagen zu kompetitieren vermag, wobei das Verfahren entweder ein Exprimieren einer entsprechenden Nukleotidsequenz, die für das Polypeptid codiert, in einer Wirtszelle oder eine chemische Peptidsynthse umfaßt.

3. Verfahren zur Herstellung eines Polypeptids mit Leukämiehemmfaktor (LIF)-Aktivität, wobei das Polypeptid
   (a) die Proliferierung von leukämischen, myeloischen M1-Zellen zu supprimieren vermag,
   (b) die in den Figuren 15, 26 oder 29 dargestellte Aminosäuresequenz entweder vollständig oder teilweise umfaßt,
   (c) mit einem Polypeptid der Sequenz um eine Bindung an spezifischen zellulären Rezeptoren auf M1-Zellen oder Mäuse- oder Humanmakrophagen zu kompetitieren vermag, und
   (d) durch eine Nukleotidsequenz codiert ist, die unter Bedingungen von 6xSSC, 65 °C oder Bedingungen höherer Strenge mit einer Sequenz eines für LIF codierenden Inserts, ausgewählt aus den für LIF codierenden Inserten der Klone pLIF7.2b, pLIFNK1, pLIFNK3 und pHGLIFBamI entspre-

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chend den Definitionen in den Figuren 10, 11, 15 oder 25, zu hybridisieren vermag, wobei das Verfahren entweder ein Exprimieren einer entsprechenden Nukleotidsequenz, die für das Polypeptid codiert, in einer Wirtszelle oder eine chemische Peptidsynthese umfaßt.

4. Verfahren zur Herstellung eines Polypeptids mit Leukämiehemmfaktor (LIF)-Aktivität, wobei das Polypeptid die Profilierung von leukämischen, myeloischen M1-Zellen zu supprimieren vermag, die in den Figuren 15, 26 oder 29 dargestellte Aminosäuresequenz entweder vollständig oder teilweise oder eine Sequenz, die eine zu mindestens 78% betragende Aminosäurehomologie damit aufweist, umfaßt, und mit einem Polypeptid der Sequenz um die Bindung an spezifische zelluläre Rezeptoren auf M1-Zellen oder Mäuse- oder Humanmakrophagen zu kompetitieren vermag, wobei das Verfahren entweder ein Exprimieren einer entsprechenden Nukleotidsequenz, die für das Polypeptid codiert, in einer Wirtszelle oder eine chemische Peptidsynthese umfaßt.

5. Verfahren zur Herstellung eines Polypeptids mit Leukämiehemmfaktor (LIF)-Aktivität, das die Proliferierung von leukämischen, myeloischen M1-Zellen zu supprimieren vermag und die in den Figuren 15, 26 oder 29 dargestellte Aminosäuresequenz umfaßt, wobei das Verfahren entweder ein Exprimieren einer entsprechenden Nukleotidsequenz, die für das Polypeptid codiert, in einer Wirtszelle oder eine chemische Peptidsynthese umfaßt.

6. Verfahren zur Reinigung eines glykosylierten oder nicht-glykosylierten LIF-Polypeptids nach Anspruch 1, umfassend
(a) das Chromatographieren von LIF in Rohform auf einer Anionenaustauschersäule und das Eluieren von LIF mit Hilfe eines zunehmenden Salzgradienten,
(b) das Chromatographieren des Produkts aus Stufe (a) auf einer Lectin-Affinitätssäule, wobei das Lectin eine Affinität für Mannose aufweist, und das Eluieren von LIF mit einem Mannosederivat,
(c) das Chromatographieren des Produkts aus Stufe (b) auf einer Kationenaustauschersäule und das Eluieren von LIF mit einem zunehmenden Salzgradienten,
(d) das Chromatographieren des Produkts aus Stufe (c) unter HPLC-Bedingungen auf einer Säule mit reverser Phase und das Eluieren von LIF mit einem zunehmenden Acetonitrilgradienten.

7. Verfahren nach Anspruch 6, wobei der LIF in Rohform ein mit Krebs II-Aszitestumorzellen konditioniertes Medium enthält und Mäuse-LIF aus diesem Medium gereinigt wird.

8. Verfahren nach Anspruch 6, wobei der LIF in Rohform ein mit einer menschlichen Blasenkarzinomzellinie 5637 (ATCC Hinterlegungsnummer HTB9) konditioniertes Medium enthält und das menschliche LIF aus diesem Medium gereinigt wird.

9. Rekombinantes DNA-Molekül mit einer Nukleotidsequenz, die für ein LIF-Polypeptid nach einem der Ansprüche 1 bis 5 codiert.

10. Rekombinantes DNA-Molekül mit einer in den Figuren 10, 15 (Basen 1-630), 25 oder 29 (Basen 1-543) vollständig oder teilweise dargestellten Nukleotidsequenz, das für ein Polypeptid mit der Fähigkeit zum Supprimieren der Profilierung von leukämischen, myeloischen M1-Zellen und zum Kompetitieren mit einem Polypeptid mit einer in den Figuren 15, 26 oder 29 dargestellten Aminosäuresequenz um die Bindung an spezifische zelluläre Rezeptoren auf M1-Zellen oder Mäuse- oder Humanmakrophagen codiert.

11. Rekombinantes DNA-Molekül mit einer Nukleotidsequenz, die bei Expression in einer geeigneten Wirtszelle für ein LIF-Polypeptid nach Anspruch 1 codiert, wobei die Nukleotidsequenz unter Bedingungen von 6xSSC, 65 °C oder Bedingungen höherer Strenge mit einer Sequenz eines für LIF codierenden Inserts, ausgewählt aus den für LIF codierenden Inserten der Klone pLIF7.2b, pLIFNK1, pLIFNK3 und pHGLIFBamI entsprechend den Definitionen in Figuren 10, 11, 15 oder 25, hybridisiert.

12. Rekombinantes DNA-Molekül mit einer Nukleotidsequenz, die bei Expression in einer geeigneten Wirtszelle für ein LIF-Polypeptide nach Anspruch 1 codiert, wobei die Nukleotidsequenz bei Expression in einem eukaryontischen Wirt für ein Polypeptid codiert, das ein reifes Polypeptid umfaßt, welches zumindest zu 78% mit einer Aminosäuresequenz eines reifen Polypeptids, ausgewählt aus den in den Figuren 15, 26 und 29 dargestellten, homolog ist.

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13. Molekül nach Anspruch 12, wobei das reife Polypeptid eine vollständige Homologie mit der in Figur 29 dargestellten Aminosäuresequenz aufweist.

14. Molekül nach einem der Ansprüche 9 bis 13, des weiteren umfassend einen Replikationsursprung, wobei das Molekül in einen zur Verwendung als Klonierungsvektor geeigneten Zustand gebracht wurde.

15. Molekül nach Anspruch 14, des weiteren umfassend eine funktionell mit der Nukleotidsequenz verknüpfte ("operably linked") Promotorsequenz, wobei das Molekül in einen zur Verwendung eines Expressionsvektors geeigneten Zustand gebracht wurde.

16. Wirtszelle mit einem rekombinanten DNA-Molekül nach Anspruch 15.

17. Verfahren zur Herstellung eines LIF-Polypeptids nach Anspruch 1, umfassend:
(a) das Bereitstellen von gemäß Anspruch 16 hergestellten Wirtszellen,
(b) das Kultivieren dieser Zellen unter Bedingungen, die die Expression des Polypeptids fördern, und
(c) das Gewinnen des Polypeptids.

18. Verfahren nach Anspruch 17, wobei das Polypeptid ein reifes Polypeptid umfaßt, das zu mindestens 78% mit einer Aminosäuresequenz, ausgewählt unter den in den Figuren 15, 26 und 29 dargestellten, homolog ist.

19. Verfahren nach Anspruch 17, wobei das reife Polypeptid eine vollständige Homologie mit der in Figur 29 dargestellten Aminosäuresequenz aufweist.

20. Verfahren nach Anspruch 17, wobei das Polypeptid durch die Wirtszelle glykosyliert ist.

21. Verfahren nach Anspruch 17, wobei die Wirtszelle aus einer Hefezelle besteht.

22. Verfahren nach Anspruch 17, wobei der Promotor aus einem galaktoseinduzierbaren Hybrid-GAL-CYC-Promotor besteht.

23. Verfahren nach Anspruch 17, wobei das Molekül des weiteren eine funktionell mit der Nukleotidsequenz verknüpfte ("operably linked") Signalsequenz umfaßt, wobei die Signalsequenz für eine Expression für eine auf die Absonderung des Polypeptids gerichtete Leadersequenz codiert.

24. Verfahren nach Anspruch 23, wobei die Signalsequenz von der Signalsequenz des Killertoxingens Kluyveromyces lactis abgeleitet ist.

25. Verfahren nach Anspruch 21, bei dem die Wirtszelle aus einer der Spezies Saccharomyces cerevisiae besteht.

26. Verfahren nach Anspruch 17, wobei die Wirtszelle aus einer Säugetierzelle besteht.

27. Verfahren nach Anspruch 26, wobei die Wirtszelle mit einem retroviralen Expressionsvektor transformiert ist.

28. Verfahren nach Anspruch 26, wobei der Vektor von dem Moloney-Mäuseleukämievirus abgeleitet ist.

29. Verfahren nach Anspruch 28, wobei der Vektor des weiteren den LTR-Verstärker eines myeloproliferativen Sarkomvirus umfaßt.

30. Verfahren nach Anspruch 29, wobei dem Vektor der nichttranslatierte 3'-Bereich natürlicher LIF-mRNA fehlt.

31. Verfahren nach Anspruch 26, wobei die Wirtszellen aus blutbildenden Zellen bestehen.

32. Verfahren nach Anspruch 17, wobei die Wirtszellen E. coli-Zellen sind.

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33. Verfahren nach Anspruch 32, wobei der Vektor aus einem besteht, der auf die Expression eines Glutathion-S-Transferase/LIF-Fusionsproteins gerichtet ist.

34. Verfahren nach Anspruch 33, wobei der Vektor aus einem besteht, der auf die Expression eines Glutathion-S-Transferase/Thrombin-Spaltungsstelle/LIF-Fusionsproteins gerichtet ist.

35. Verfahren zur Herstellung eines LIF-Polypeptids nach Anspruch 1, wobei das Polypeptid eine Aminosäuresequenz einschließlich den Aminosäureresten 179-187 von natürlichem Mäuse-LIF gemäß Figur 15 aufweist.

36. Verfahren nach Anspruch 35, wobei das Polypeptid einen N-Terminus aufweist, dem mindestens die ersten drei Aminosäurereste natürlicher Mäuse-LIF fehlen.

37. Verwendung eines LIF-Polypeptids mit LIF-Aktivität nach einem der Ansprüche 1 bis 5 und 35 bis 37 zur Herstellung einer Zubereitung zur Hemmung der Profilierung von leukämischen myeloischen Zellen

38. Verwendung nach Anspruch 37 zur Herstellung einer Zubereitung, des weiteren umfassend ein Polypeptid mit G-CSF- oder GM-CSF-Aktivität.

39. Verfahren zur Herstellung einer pharmazeutischen Zubereitung, umfassend ein LIF-Polypeptid nach einem der Ansprüche 1 bis 5 und 35 bis 37, umfassend das Vermischen des Polypeptids mit einem oder mehreren pharmazeutisch akzeptablen Trägern und/oder Verdünnungsmitteln.

40. Verfahren nach Anspruch 39, wobei das reife LIF-Polypeptid vollständig mit der in Fig. 29 dargestellten Aminosäuresequenz homolog ist.

41. Verfahren nach Anspruch 39 oder Anspruch 40, des weiteren umfassend das Vermischen mit mindestens einem weiteren biologischen Regulator von Blutzellen.

42. Verfahren nach Anspruch 41, wobei der weitere Regulator aus einem Polypeptid mit G-CSF- oder GM-CSF-Aktivität besteht.

Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, LI, DE, FR, GB, IT, LU, NL, SE

1. Polypeptide présentant une activité de facteur inhibiteur de leucémie (LIF) caractérisé en ce qu'il est capable de supprimer la prolifération de cellules leucémiques myéloïdes M1, en ce qu'il comprend la séquence d'aminoacides indiquée sur les figures 15, 26 ou 29, complètement ou en partie, et en ce qu'il est capable d'entrer en compétition avec un polypeptide de ladite séquence pour une liaison à des récepteurs cellulaires spécifiques de cellules M1 ou de macrophages murins ou humains.

2. Polypeptide présentant une activité de facteur inhibiteur de leucémie (LIF), caractérisé en ce qu'il est capable de supprimer la prolifération de cellules leucémiques myéloïdes M1, en ce qu'il est codé par une séquence nucléotidique qui est capable de s'hybrider, dans des conditions de 6xSSC, 65 °C ou dans des conditions de plus grande rigueur, à une séquence d'un insert codant LIF choisi parmi les inserts codant LIF de clones pLIF7.2b, pLIFNK1, pLIFNK3 et pHGLIFBamI, tels que définis dans les figurez 10, 11, 15 ou 25, et en ce qu'il est capable d'entrer en compétition avec un polypeptide de la séquence d'aminoacides indiquée sur les figures 15, 26 ou 29 pour une liaison à des récepteurs cellulaires spécifiques de cellules M1 ou de macrophages murins ou humains.

3. Polypeptide présentant une activité de facteur inhibiteur de leucémie (LIF), caractérisé en ce que :
   (a) il est capable de supprimer la prolifération de cellules leucémiques myéloïdes M1,
   (b) il comprend la séquence d'aminoacides indiquée dans les figures 15, 26 ou 29, complètement ou en partie,
   (c) il est capable d'entrer en compétition avec un polypeptide de cette séquence pour une liaison à des récepteurs cellulaires spécifiques de cellules M1 ou de macrophages murins ou humains, et
   (d) il est codé par une séquence nucléotidique qui est capable de s'hybrider, dans des conditions de 6xSSC, 65 °C ou des conditions de plus grande rigueur, à une séquence d'un insert codant LIF,

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choisi parmi les inserts codant LIF de clones pLIF7.2b, pLIFNK1, pLIFNK3 et pHGLIFBamI, tels que définis dans les figures 10, 11, 15 ou 25.

4. Polypeptide présentant une activité de facteur inhibiteur de leucémie (LIF), caractérisé en ce qu'il est capable de supprimer la prolifération de cellules leucémiques myéloïdes, en ce qu'il comprend la séquence d'aminoacides indiquée dans les figures 15, 26 ou 29, complètement ou en partie, ou une séquence qui a au moins une homologie de 78 % des aminoacides avec elle, et en ce qu'il est capable d'entrer en compétition avec un polypeptide de ladite séquence pour une liaison à des récepteurs cellulaires spécifiques de cellules M1 ou de macrophages murins ou humains.

5. Polypeptide présentant une activité de facteur inhibiteur de leucémie (LIF), qui est capable de supprimer la prolifération de cellules leucémiques myéloïdes M1 et qui comprend la séquence d'aminoacides indiquée sur les figures 15, 26 ou 29.

6. Procédé de purification d'un polypeptide LIF suivant la revendication 1, glycosylé ou non glycosylé, comprenant (a) une chromatographie de LIF sous forme brute sur une colonne échangeuse d'anions, et une élution de LIF par un gradient de sel croissant, (b) une chromatographie du produit de l'étape (a) sur une colonne d'affinité à base de lectine, cette lectine ayant une affinité pour du mannose, et une élution de LIF par un dérivé de mannose, (c) une chromatographie du produit de l'étape (b) sur une colonne échangeuse de cations et une élution de LIF par un gradient de sel croissant, (d) une chromatographie du produit de l'étape (c) dans des conditions HPLC sur une colonne à phase inverse et une élution de LIF par un gradient croissant d'acétonitrile.

7. Procédé suivant la revendication 6, caractérisé en ce que le LIF sous forme brute comprend un milieu conditionné de cellules tumorales d'ascites de Krebs II, et en ce que le LIF murin est purifié à partir de ce milieu.

8. Procédé suivant la revendication 6, caractérisé en ce que le LIF sous forme brute comprend un milieu conditionné d'une lignée cellulaire 5637 de carcinome de vessie humain (ATCC dépôt n° HTB9), et en ce que le LIF humain est purifié à partir de ce milieu.

9. Molécule d'ADN recombinant comprenant une séquence nucléotidique qui code pour une polypeptide LIF tel que défini dans l'une quelconque des revendications 1 à 5.

10. Molécule d'ADN recombinant comprenant une séquence nucléotidique telle qu'indiquée dans les figures 10, 15 (bases 1-630), 25 ou 29 (bases 1-543), complètement ou en partie, qui code un polypeptide capable de supprimer la prolifération de cellules leucémiques myéloïdes M1 et d'entrer en compétition avec un polypeptide ayant une séquence d'aminoacides indiquée dans les figures 15, 26 ou 29 pour une liaison à des récepteurs cellulaires spécifiques de cellules M1 ou de macrophages murins ou humains.

11. Molécule d'ADN recombinant comprenant une séquence nucléotidique qui, en expression dans une cellule hôte appropriée, code pour un polypeptide LIF tel que défini dans la revendication 1, cette séquence nucléotidique s'hybridant, dans des conditions de 6xSSC, 65 °C ou des conditions de plus grande rigueur, à une séquence d'un insert codant LIF choisi parmi les inserts codant LIF de clones pLIF7.2b, pLIFNK1, pLIFNK3 et pHGLIFBamI, tels que définis sur les figures 10, 11, 15 ou 25.

12. Molécule d'ADN recombinant comprenant une séquence nucléotidique qui, en expression dans une cellule hôte appropriée, code pour un polypeptide LIF tel que défini dans la revendication 1, la séquence nucléotidique, en expression dans un hôte eucaryote, codant pour un polypeptide qui comprend un polypeptide à maturité qui est homologue à au moins 78 % avec une séquence d'aminoacides de polypeptide à maturité choisie parmi celles indiquées dans les figures 15, 26 et 29.

13. Molécule suivant la revendication 12, caractérisée en ce que le polypeptide à maturité présente une totale homologie avec la séquence d'aminoacides indiquée dans la figure 29.

14. Molécule suivant l'une quelconque des revendications 9 à 13, comprenant en outre une origine de réplication, la molécule étant rendue appropriée à une utilisation comme vecteur de clonage.

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15. Molécule suivant la revendication 14, comprenant en outre une séquence de promoteur reliée de manière fonctionnelle à ladite séquence nucléotidique, cette molécule étant rendue appropriée à une utilisation comme vecteur d'expression.

16. Cellule hôte contenant une molécule d'ADN recombinant suivant la revendication 15.

17. Procédé de préparation d'un polypeptide LIF suivant la revendication 1, comprenant : (a) une fourniture de cellules hôtes contenant une molécule suivant la revendication 14, (b) une culture de ces cellules dans des conditions favorables à une expression de ce polypeptide, et (c) une récupération de ce polypeptide.

18. Procédé suivant la revendication 17, caractérisé en ce que le polypeptide comprend un polypeptide à maturité qui est homologue pour au moins 78 % à une séquence d'aminoacides choisie parmi celles indiquées dans les figurez 15, 26 et 29.

19. Procédé suivant la revendication 17, caractérisé en ce que le polypeptide à maturité présente une totale homologie avec la séquence d'aminoacides indiquée dans la figure 29.

20. Procédé suivant la revendication 17, caractérisé en ce que le polypeptide est glycosylé par la cellule hôte.

21. Procédé suivant la revendication 17, caractérisé en ce que la cellule hôte est une cellule de levure.

22. Procédé suivant la revendication 17, caractérisé en ce que le promoteur est un promoteur GAL-CYC hybride inductible par du galactose.

23. Procédé suivant la revendication 17, caractérisé en ce que la molécule comprend en outre une séquence de signal reliée de manière fonctionnelle à ladite séquence nucléotidique, cette séquence de signal codant une expression pour une séquence conductrice dirigeant la sécrétion du polypeptide.

24. Procédé suivant la revendication 23, caractérisé en ce que la séquence de signal est dérivée de la séquence de signal du gène de toxine meurtrière de Kluyveromyces lactis.

25. Procédé suivant la revendication 21, caractérisé en ce que la cellule hôte est de l'espèce Saccharomyces cerevisiae.

26. Procédé suivant la revendication 17, caractérisé en ce que la cellule hôte est une cellule de mammifère.

27. Procédé suivant la revendication 26, caractérisé en ce que la cellule hôte est transformée par un vecteur d'expression rétroviral.

28. Procédé suivant la revendication 26, caractérisé en ce que le vecteur est dérivé du virus de leucémie de murine Moloney.

29. Procédé suivant la revendication 28, caractérisé en ce que le vecteur comprend en outre l'agent d'activation LTR du virus du sarcome myéloprolifératoeur.

30. Procédé suivant la revendication 29, caractérisé en ce que le vecteur est dénué de la région non traduite 3' de l'ARNm de LIF natif.

31. Procédé suivant la revendication 26, caractérisé en ce que les cellules hôtes sont des cellules hématopoïétiques.

32. Procédé suivant la revendication 17, caractérisé en ce que les cellules hôtes sont des cellules de E.coli.

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33. Procédé suivant la revendication 32, caractérisé en ce que le vecteur est un vecteur qui dirige l'expression d'une protéine de fusion de glutathion S-transférase/LIF.

34. Procédé suivant la revendication 33, caractérisé en ce que le vecteur est un vecteur qui dirige l'expression d'une protéine de fusion de glutathion S-transférase/site de clivage de thrombine/LIF.

35. Polypeptide LIF, qui peut être obtenu par le procédé de la revendication 17.

36. Polypeptide LIF suivant la revendication 1, qui présente une séquence d'aminoacides contenant des radicaux 179-187 d'aminoacides de LIF murin naturel, comme indiqué sur la figure 15.

37. Polypeptide suivant la revendication 36, comportant un terminal en N qui est dénué d'au moins les trois premiers radicaux d'aminoacide du LIF murin naturel.

38. Utilisation d'un polypeptide LIF présentant une activité de LIF suivant l'une quelconque des revendications 1 à 5 et 35 à 37, pour la préparation d'une composition destinée à empêcher la prolifération de cellules leucémiques myéloïdes.

39. Utilisation suivant la revendication 38, pour la préparation d'une composition comprenant en outre un polypeptide présentant une activité de G-CSF ou GM-CSF.

40. Composition pharmaceutique comprenant un polypeptide LIF suivant l'une quelconque des revendications 1 à 5 et 35 à 37, en association avec un ou plusieurs supports et/ou diluants pharmaceutiquement acceptables.

41. Composition suivant la revendication 40, caractérisée en ce que le polypeptide LIF à maturité est totalement homologue à la séquence d'aminoacides indiquée sur la figure 29.

42. Composition suivant l'une des revendications 40 et 41, comprenant en outre au moins un autre régulateur biologique de cellules sanguines.

43. Composition suivant la revendication 42, caractérisée en ce que ledit autre régulateur est un polypeptide présentant une activité de G-CSF ou GM-CSF.

44. Sonde ou réactif de diagnostic comprenant au moins une séquence d'acides nucléiques correspondant à une séquence ou sous-séquence d'un insert codant un polypeptide LIF, choisi parmi le groupe comprenant les inserts codant LIF de clones pLIF7.2b, pLIFNK1, pLIFNK3 et pHGLIFBamI, tels que définis sur les figures 10, 11, 15 ou 25, ou à une séquence ou sousséquence des ARNm correspondants.

Revendications pour l'Etat contractant suivant : ES

1. Procédé de préparation d'un polypeptide présentant une activité de facteur inhibiteur de leucémie (LIF), dans lequel le polypeptide est capable de supprimer la prolifération de cellules leucémiques myéloïdes M1, comprend la séquence d'aminoacides indiquée sur les figures 15, 26 ou 29, complètement ou en partie, et est capable d'entrer en compétition avec un polypeptide de ladite séquence pour une liaison à des récepteurs cellulaires spécifiques de cellules M1 ou de macrophages murins ou humains, ce procédé comprenant une expression dans une cellule hôte d'une séquence nucléotidique correspondant codant ledit polypeptide ou une synthèse de peptide chimique.

2. Procédé de préparation d'un polypeptide présentant une activité de facteur inhibiteur de leucémie (LIF), dans lequel le polypeptide est capable de supprimer la prolifération de cellules leucémiques myéloïdes M1, est codé par une séquence nucléotidique qui est capable de s'hybrider, dans des conditions de 6xSSC, 65 °C ou dans des conditions de plus grande rigueur, à une séquence d'un insert codant LIF choisi parmi les inserts codant LIF de clones pLIF7.2b, pLIFNK1, pLIFNK3 et pHGLIFBamI, tels que définis dans les figures 10, 11, 15 ou 25, et est capable d'entrer en compétition avec un polypeptide de la séquence d'aminoacides indiquée sur les figures 15, 26 ou 29 pour une liaison à des récepteurs cellulaires spécifiques de cellules M1 ou de macrophages murins ou humains, ce procédé comprenant

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une expression dans une cellule hôte d'une séquence nucléotidique correspondante codant ledit polypeptide ou une synthèse de peptide chimique.

3. Procédé de préparation d'un polypeptide présentant une activité de facteur inhibiteur de leucémie (LIF), dans lequel le polypeptide
(a) est capable de supprimer la prolifération des cellules leucémiques myéloïdes M1,
(b) comprend la séquence d'aminoacides indiquée dans les figures 15, 26 ou 29, complètement ou en partie,
(c) est capable d'entrer en compétition avec un polypeptide de ladite séquence pour une liaison à des récepteurs cellulaires spécifiques de cellules M1 ou de macrophages murins ou humains, et
(d) est codé par une séquence nucléotidique qui est capable de s'hybrider, dans des conditions de 6xSSC, 65 °C ou des conditions de plus grande rigueur, à une séquence d'un insert codant LIF choisi parmi les inserts codant LIF de clones pLIF7.2b, pLIFNK1, pLIFNK3 et pHGLIFBamI, tels que définis dans les figures 10, 11, 15 ou 25, ce procédé comprenant une expression dans une cellule hôte d'une séquence nucléotidique correspondante codant ledit polypeptide ou une synthèse de peptide chimique.

4. Procédé de préparation d'un polypeptide présentant une activité de facteur inhibiteur de leucémie (LIF), dans lequel le polypeptide est capable de supprimer la prolifération de cellules leucémiques myéloïdes M1, comprend la séquence d'aminoacides indiquée sur les figures 15, 26 ou 29, complètement ou en partie, ou une séquence qui a au moins une homologie de 78 % des aminoacides avec elle, et est capable d'entrer en compétition avec un polypeptide de ladite séquence pour une liaison à des récepteurs cellulaires spécifiques de cellules M1 ou de macrophages murins ou humains, ce procédé comprenant une expression dans une cellule hôte d'une séquence nucléotidique correspondante codant ledit polypeptide ou une synthèse de peptide chimique.

5. Procédé de préparation d'un polypeptide présentant une activité de facteur inhibiteur de leucémie (LIF) qui est capable de supprimer la prolifération de cellules leucémiques myéloïdes M1 et qui comprend la séquence d'aminoacides indiquée sur les figures 15, 26 ou 29, ce procédé comprenant une expression dans une cellule hôte d'une séquence nucléotidique correspondante codant ledit polypeptide ou une synthèse de peptide chimique.

6. Procédé de purification d'un polypeptide LIF suivant la revendication 1, glycosylé ou non glycosylé, comprenant (a) une chromatographie de LIF sous forme brute sur une colonne échangeuse d'anions, et une élution de LIF par un gradient de sel croissant, (b) une chromatographie du produit de l'étape (a) sur une colonne d'affinité à base de lectine, cette lectine ayant une affinité pour du mannose, et une élution de LIF par un dérivé de mannose, (c) une chromatographie du produit de l'étape (b) sur une colonne échangeuse de cations et une élution de LIF par un gradient de sel croissant, (d) une chromatographie du produit de l'étape (c) dans des conditions HPLC sur une colonne à phase inverse et une élution de LIF par un gradient croissant d'acétonitrile.

7. Procédé suivant la revendication 6, caractérisé en ce que le LIF sous forme brute comprend un milieu conditionné de cellules tumorales d'ascites de Krebs II, et en ce que le LIF murin est purifié à partir de ce milieu.

8. Procédé suivant la revendication 6, caractérisé en ce que le LIF sous forme brute comprend un milieu conditionné d'une lignée cellulaire 5637 de carcinome de vessie humain (ATCC dépôt n° HTB9), et en ce que le LIF humain est purifié à partir de ce milieu.

9. Procédé de préparation d'une molécule d'ADN recombinant comprenant une séquence nucléotidique qui code pour un polypeptide LIF suivant l'une quelconque des revendications 1 à 5, ce procédé comprenant l'étape d'insertion de la séquence nucléotidique dans un ADN vecteur.

10. Procédé de préparation d'une molécule d'ADN recombinant comprenant une séquence nucléotidique, telle qu'indiquée sur les figures 10, 15 (bases 1-630), 25 ou 29 (bases 1-543), complètement ou en partie, qui code un polypeptide capable de supprimer la prolifération de cellules leucémiques myéloïdes M1 et entre en compétition avec un polypeptide ayant une séquence d'aminoacides indiquée sur les figures 15, 26 ou 29, pour une liaison à des récepteurs cellulaires spécifiques de cellules M1 ou de

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macrophages murins ou humains, ce procédé comprenant l'étape d'insertion de la séquence nucléotidique dans un ADN vecteur.

11. Procédé de préparation d'une molécule d'ADN recombinant comprenant une séquence nucléotidique qui, en expression dans une cellule hôte appropriée, code pour un polypeptide LIF suivant la revendication 1, ladite séquence nucléotidique s'hybridant, dans des conditions 6xSSC, 65 °C ou des conditions de plus grande rigueur, à une séquence d'un insert codant LIF choisi parmi les inserts codant LIF de clones pLIF7.2b, pLIFNK1, pLIFNK3 et pHGLIFBamI, tels que définis sur les figures 10, 11, 15 ou 25, ce procédé comprenant l'étape d'insertion de la séquence nucléotidique dans un ADN vecteur.

12. Procédé de préparation d'une molécule d'ADN recombinant comprenant une séquence nucléotidique qui, en expression dans une cellule hôte appropriée, code pour un polypeptide LIF suivant la revendication 1, la séquence nucléotidique, en expression dans un hôte eucaryote, codant pour un polypeptide qui comprend un polypeptide à maturité qui est au moins à 78 % homologue à une séquence d'aminoacides de polypeptide à maturité choisie parmi celles indiquées sur les figures 15, 26 et 29, ce procédé comprenant l'étape d'insertion de la séquence nucléotidique dans un ADN vecteur.

13. Procédé suivant la revendication 12, caractérisé en ce que le polypeptide à maturité présente une totale homologie avec la séquence d'aminoacides indiquée sur la figure 29.

14. Procédé suivant l'une quelconque des revendications 9 à 13, comprenant en outre une insertion d'une origine de réplication, ladite molécule étant rendue appropriée à une utilisation comme vecteur de clonage.

15. Procédé suivant la revendication 14, comprenant en outre une insertion d'une séquence de promoteur reliée de manière fonctionnelle à ladite séquence nucléotidique, ladite molécule étant rendue appropriée à une utilisation comme vecteur d'expression.

16. Procédé de préparation d'une cellule hôte capable d'exprimer un polypeptide LIF suivant l'une quelconque des revendications précédentes, comprenant une introduction dans la cellule hôte d'une molécule d'ADN recombinant telle que définie dans la revendication 15.

17. Procédé de production d'un polypeptide LIF suivant la revendication 1, comprenant (a) la fourniture de cellules hôtes préparées suivant la revendication 16, (b) une culture de ces cellules dans des conditions favorables à une expression du polypeptide, et (c) une récupération de ce polypeptide.

18. Procédé suivant la revendication 17, caractérisé en ce que le polypeptide comprend un polypeptide à maturité qui est homologue pour au moins 78 % à une séquence d'aminoacides choisie parmi celles indiquées dans les figures 15, 26 et 29.

19. Procédé suivant la revendication 17, caractérisé en ce que le polypeptide à maturité présente une totale homologie avec la séquence d'aminoacides indiquée dans la figure 29.

20. Procédé suivant la revendication 17, caractérisé en ce que le polypeptide est glycosylé par la cellule hôte.

21. Procédé suivant la revendication 17, caractérisé en ce que la cellule hôte est une cellule de levure.

22. Procédé suivant la revendication 17, caractérisé en ce que le promoteur est un promoteur GAL-CYC hybride inductible par du galactose.

23. Procédé suivant la revendication 17, caractérisé en ce que la molécule comprend en outre une séquence de signal reliée de manière fonctionnelle à ladite séquence nucléotidique, cette séquence de signal codant une expression pour une séquence conductrice dirigeant la sécrétion du polypeptide.

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24. Procédé suivant la revendication 23, caractérisé en ce que la séquence de signal est dérivée de la séquence de signal du gène de toxine meurtrière de Kluyveromyces lactis.

25. Procédé suivant la revendication 21, caractérisé en ce que la cellule hôte est de l'espèce Saccharomyces cerevisiae.

26. Procédé suivant la revendication 17, caractérisé en ce que la cellule hôte est une cellule de mammifère.

27. Procédé suivant la revendication 26, caractérisé en ce que la cellule hôte est transformée par un vecteur d'expression rétroviral.

28. Procédé suivant la revendication 26, caractérisé en ce que le vecteur est dérivé du virus de leucémie de murine Moloney.

29. Procédé suivant la revendication 28, caractérisé en ce que le vecteur comprend en outre l'agent d'activation LTR du virus du sarcome myéloprolifératéur.

30. Procédé suivant la revendication 29, caractérisé en ce que le vecteur est dénué de la région non traduite 3' de l'ARNm de LIF natif.

31. Procédé suivant la revendication 26, caractérisé en ce que les cellules hôtes sont des cellules hématopoïétiques.

32. Procédé suivant la revendication 17, caractérisé en ce que les cellules hôtes sont des cellules de E.coli.

33. Procédé suivant la revendication 32, caractérisé en ce que le vecteur est un vecteur qui dirige l'expression d'une protéine de fusion de glutathion S-transférase/LIF.

34. Procédé suivant la revendication 33, caractérisé en ce que le vecteur est un vecteur qui dirige l'expression d'une protéine de fusion de glutathion S-transférase/site de clivage de thrombine-LIF.

35. Procédé de préparation d'un polypeptide LIF suivant la revendication 1, caractérisé en ce que le polypeptide présente une séquence d'aminoacides contenant des radicaux 179-187 d'aminoacides du LIF murin naturel, tel qu'indiqué sur la figure 15.

36. Procédé suivant la revendication 35, caractérisé en ce que le polypeptide présente un terminal en N qui est dénué d'au moins les trois premiers radicaux aminoacides du LIF murin naturel.

37. Utilisation d'un polypeptide de LIF présentant une activité de LIF suivant l'une quelconque des revendications 1 à 5 et 35 à 36, pour la préparation d'une composition destinée à empêcher la prolifération de cellules leucémiques myéloïdes.

38. Utilisation suivant la revendication 38, pour la préparation d'une composition comprenant en outre un polypeptide présentant une activité de G-CSF ou GM-CSF.

39. Procédé de préparation d'une composition pharmaceutique comprenant un polypeptide LIF suivant l'une quelconque des revendications 1 à 5 et 35 à 36, comprenant un mélange du polypeptide à un ou plusieurs supports et/ou diluants pharmaceutiquement acceptables.

40. Procédé suivant la revendication 39, caractérisé en ce que le polypeptide LIF à maturité est totalement homologue à la séquence d'aminoacides indiquée sur la figure 29.

41. Procédé suivant l'une des revendications 39 et 40, comprenant en outre un mélange d'au moins un autre régulateur biologique de cellules sanguines.

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42. Procédé suivant la revendication 41, caractérisé en ce que l'autre régulateur est un polypeptide ayant une activité de G-CSF ou GM-CSF.

Revendications pour l'Etat contractant suivant : GR

1. Procédé de préparation d'un polypeptide présentant une activité de facteur inhibiteur de leucémie (LIF), dans lequel le polypeptide est capable de supprimer la prolifération de cellules leucémiques myéloïdes M1, comprend la séquence d'aminoacides indiquée sur les figures 15, 26 ou 29, complètement ou en partie, et est capable d'entrer en compétition avec un polypeptide de ladite séquence pour une liaison à des récepteurs cellulaires spécifiques de cellules M1 ou de macrophages murins ou humains, ce procédé comprenant une expression dans une cellule hôte d'une séquence nucléotidique correspondant codant ledit polypeptide ou une synthèse de peptide chimique.

2. Procédé de préparation d'un polypeptide présentant une activité de facteur inhibiteur de leucémie (LIF), dans lequel le polypeptide est capable de supprimer la prolifération de cellules leucémiques myéloïdes M1, est codé par une séquence nucléotidique qui est capable de s'hybrider, dans des conditions de 6xSSC, 65 °C ou dans des conditions de plus grande rigueur, à une séquence d'un insert codant LIF choisi parmi les inserts codant LIF de clones pLIF7.2b, pLIFNK1, pLIFNK3 et pHGLIFBamI, tels que définis dans les figures 10, 11, 15 ou 25, et est capable d'entrer en compétition avec un polypeptide de la séquence d'aminoacides indiquée sur les figures 15, 26 ou 29 pour une liaison à des récepteurs cellulaires spécifiques de cellules M1 ou de macrophages murins ou humains, ce procédé comprenant une expression dans une cellule hôte d'une séquence nucléotidique correspondante codant ledit polypeptide ou une synthèse de peptide chimique.

3. Procédé de préparation d'un polypeptide présentant une activité de facteur inhibiteur de leucémie (LIF), dans lequel le polypeptide
   (a) est capable de supprimer la prolifération des cellules leucémiques myéloïdes M1,
   (b) comprend la séquence d'aminoacides indiquée dans les figures 15, 26 ou 29, complètement ou en partie,
   (c) est capable d'entrer en compétition avec un polypeptide de ladite séquence pour une liaison à des récepteurs cellulaires spécifiques de cellules M1 ou de macrophages murins ou humains, et
   (d) est codé par une séquence nucléotidique qui est capable de s'hybrider, dans des conditions de 6xSSC, 65 °C ou des conditions de plus grande rigueur, à une séquence d'un insert codant LIF choisi parmi les inserts codant LIF de clones pLIF7.2b, pLIFNK1, pLIFNK3 et pHGLIFBamI, tels que définis dans les figures 10, 11, 15 ou 25, ce procédé comprenant une expression dans une cellule hôte d'une séquence nucléotidique correspondante codant ledit polypeptide ou une synthèse de peptide chimique.

4. Procédé de préparation d'un polypeptide présentant une activité de facteur inhibiteur de leucémie (LIF), dans lequel le polypeptide est capable de supprimer la prolifération de cellules leucémiques myéloïdes M1, comprend la séquence d'aminoacides indiquée sur les figures 15, 26 ou 29, complètement ou en partie, ou une séquence qui a au moins une homologie de 78 % des aminoacides avec elle, et est capable d'entrer en compétition avec un polypeptide de ladite séquence pour une liaison à des récepteurs cellulaires spécifiques de cellules M1 ou de macrophages murins ou humains, ce procédé comprenant une expression dans une cellule hôte d'une séquence nucléotidique correspondante codant ledit polypeptide ou une synthèse de peptide chimique.

5. Procédé de préparation d'un polypeptide présentant une activité de facteur inhibiteur de leucémie (LIF) qui est capable de supprimer la prolifération de cellules leucémiques myéloïdes M1 et qui comprend la séquence d'aminoacides indiquée sur les figures 15, 26 ou 29, ce procédé comprenant une expression dans une cellule hôte d'une séquence nucléotidique correspondante codant ledit polypeptide ou une synthèse de peptide chimique.

6. Procédé de purification d'un polypeptide LIF suivant la revendication 1, glycosylé ou non glycosylé, comprenant (a) une chromatographie de LIF sous forme brute sur une colonne échangeuse d'anions, et une élution de LIF par un gradient de sel croissant, (b) une chromatographie du produit de l'étape (a) sur une colonne d'affinité à base de lectine, cette lectine ayant une affinité pour du mannose, et une élution de LIF par un dérivé de mannose, (c) une chromatographie du produit de l'étape (b) sur une

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colonne échangeuse de cations et une élution de LIF par un gradient de sel croissant, (d) une chromatographie du produit de l'étape (c) dans des conditions HPLC sur une colonne à phase inverse et une élution de LIF par un gradient croissant d'acétonitrile.

7. Procédé suivant la revendication 6, caractérisé en ce que le LIF sous forme brute comprend un milieu conditionné de cellules tumorales d'ascites de Krebs II, et en ce que le LIF murin est purifié à partir de ce milieu.

8. Procédé suivant la revendication 6, caractérisé en ce que le LIF sous forme brute comprend un milieu conditionné d'une lignée cellulaire 5637 de carcinome de vessie humain (ATCC dépôt n° HTB9), et en ce que le LIF humain est purifié à partir de ce milieu.

9. Molécule d'ADN recombinant comprenant une séquence nucléotidique qui code pour une polypeptide LIF tel que défini dans l'une quelconque des revendications 1 à 5.

10. Molécule d'ADN recombinant comprenant une séquence nucléotidique telle qu'indiquée dans les figures 10, 15 (bases 1-630), 25 ou 29 (bases 1-543), complètement ou en partie, qui code un polypeptide capable de supprimer la prolifération de cellules leucémiques myéloïdes M1 et d'entrer en compétition avec un polypeptide ayant une séquence d'aminoacides indiquée dans les figures 15, 26 ou 29 pour une liaison à des récepteurs cellulaires spécifiques de cellules M1 ou de macrophages murins ou humains.

11. Molécule d'ADN recombinant comprenant une séquence nucléotidique qui, en expression dans une cellule hôte appropriée, code pour un polypeptide LIF tel que défini dans la revendication 1, cette séquence nucléotidique s'hybridant, dans des conditions de 6xSSC, 65 °C ou des conditions de plus grande rigueur, à une séquence d'un insert codant LIF choisi parmi les inserts codant LIF de clones pLIF7.2b, pLIFNK1, pLIFNK3 et pHGLIFBamI, tels que définis sur les figures 10, 11, 15 ou 25.

12. Molécule d'ADN recombinant comprenant une séquence nucléotidique qui, en expression dans une cellule hôte appropriée, code pour un polypeptide LIF tel que défini dans la revendication 1, la séquence nucléotidique, en expression dans un hôte eucaryote, codant pour un polypeptide qui comprend un polypeptide à maturité qui est homologue à au moins 78 % avec une séquence d'aminoacides de polypeptide à maturité choisie parmi celles indiquées dans les figures 15, 26 et 29.

13. Molécule suivant la revendication 12, caractérisée en ce que le polypeptide à maturité présente une totale homologie avec la séquence d'aminoacides indiquée dans la figure 29.

14. Molécule suivant l'une quelconque des revendications 9 à 13, comprenant en outre une origine de réplication, la molécule étant rendue appropriée à une utilisation comme vecteur de clonage.

15. Molécule suivant la revendication 14, comprenant en outre une séquence de promoteur reliée de manière fonctionnelle à ladite séquence nucléotidique, cette molécule étant rendue appropriée à une utilisation comme vecteur d'expression.

16. Cellule hôte contenant une molécule d'ADN recombinant suivant la revendication 15.

17. Procédé de préparation d'un polypeptide LIF suivant la revendication 1, comprenant : (a) une fourniture de cellules hôtes contenant une molécule suivant la revendication 14, (b) une culture de ces cellules dans des conditions favorables à une expression de ce polypeptide, et (c) une récupération de ce polypeptide.

18. Procédé suivant la revendication 17, caractérisé en ce que le polypeptide comprend un polypeptide à maturité qui est homologue pour au moins 78 % à une séquence d'aminoacides choisie parmi celles indiquées dans les figures 15, 26 et 29.

19. Procédé suivant la revendication 17, caractérisé en ce que le polypeptide à maturité présente une totale homologie avec la séquence d'aminoacides indiquée dans la figure 29.

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20. Procédé suivant la revendication 17, caractérisé en ce que le polypeptide est glycosylé par la cellule hôte.

21. Procédé suivant la revendication 17, caractérisé en ce que la cellule hôte est une cellule de levure.

22. Procédé suivant la revendication 17, caractérisé en ce que le promoteur est un promoteur GAL-CYC hybride inductible par du galactose.

23. Procédé suivant la revendication 17, caractérisé en ce que la molécule comprend en outre une séquence de signal reliée de manière fonctionnelle à ladite séquence nucléotidique, cette séquence de signal codant une expression pour une séquence conductrice dirigeant la sécrétion du polypeptide.

24. Procédé suivant la revendication 23, caractérisé en ce que la séquence de signal est dérivée de la séquence de signal du gêne de toxine meurtrière de Kluyveromyces lactis.

25. Procédé suivant la revendication 21, caractérisé en ce que la cellule hôte est de l'espèce Saccharomyces cerevisiae.

26. Procédé suivant la revendication 17, caractérisé en ce que la cellule hôte est une cellule de mammifère.

27. Procédé suivant la revendication 26, caractérisé en ce que la cellule hôte est transformée par un vecteur d'expression rétroviral.

28. Procédé suivant la revendication 26, caractérisé en ce que le vecteur est dérivé du virus de leucémie de murine Moloney.

29. Procédé suivant la revendication 28, caractérisé en ce que le vecteur comprend en outre l'agent d'activation LTR du virus du sarcome myéloprolifératéur.

30. Procédé suivant la revendication 29, caractérisé en ce que le vecteur est dénué de la région non traduite 3' de l'ARNm de LIF natif.

31. Procédé suivant la revendication 26, caractérisé en ce que les cellules hôtes sont des cellules hématopoïétiques.

32. Procédé suivant la revendication 17, caractérisé en ce que les cellules hôtes sont des cellules de E.coli.

33. Procédé suivant la revendication 32, caractérisé en ce que le vecteur est un vecteur qui dirige l'expression d'une protéine de fusion de glutathion S-transférase/LIF.

34. Procédé suivant la revendication 33, caractérisé en ce que le vecteur est un vecteur qui dirige l'expression d'une protéine de fusion de glutathion S-transférase/site de clivage de thrombine/LIF.

35. Procédé de préparation d'un polypeptide LIF suivant la revendication 1, caractérisé en ce que le polypeptide présente une séquence d'aminoacides contenant des radicaux 179-187 d'aminoacides du LIF murin naturel, tel qu'indiqué sur la figure 15.

36. Procédé suivant la revendication 35, caractérisé en ce que le polypeptide présente un terminal en N qui est dénué d'au moins les trois premiers radicaux aminoacides du LIF murin naturel.

37. Utilisation d'un polypeptide de LIF présentant une activité de LIF suivant l'une quelconque des revendications 1 à 5 et 35 à 36, pour la préparation d'une composition destinée à empêcher la prolifération de cellules leucémiques myéloïdes.

38. Utilisation suivant la revendication 38, pour la préparation d'une composition comprenant en outre un polypeptide présentant une activité de G-CSF ou GM-CSF.

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39. Procédé de préparation d'une composition pharmaceutique comprenant un polypeptide LIF suivant l'une quelconque des revendications 1 à 5 et 35 à 36, comprenant un mélange du polypeptide à un ou plusieurs supports et/ou diluants pharmaceutiquement acceptables.

40. Procédé suivant la revendication 39, caractérisé en ce que le polypeptide LIF à maturité est totalement homologue à la séquence d'aminoacides indiquée sur la figure 29.

41. Procédé suivant l'une des revendications 39 et 40, comprenant en outre un mélange d'au moins un autre régulateur biologique de cellules sanguines.

42. Procédé suivant la revendication 41, caractérisé en ce que l'autre régulateur est un polypeptide ayant une activité de G-CSF ou GM-CSF.

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