PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number: WO 92/18628
C12N 15/12, C07K 13/00 G01N 33/58, 33/68, C07K 15/00	A1	(43) International Publication Date: 29 October 1992 (29.10.92)

(21) International Application Number: PCT/US92/03026

(22) International Filing Date: 16 April 1992 (16.04.92)

(30) Priority data:
688,355 19 April 1991 (19.04.91) US

(60) Parent Application or Grant
(63) Related by Continuation
US 688,355 (CON)
Filed on 19 April 1991 (19.04.91)

(71) Applicant *(for all designated States except US):* SCHERING CORPORATION [US/US]; 2000 Galloping Hill Road, Kenilworth, NJ 07033 (US).

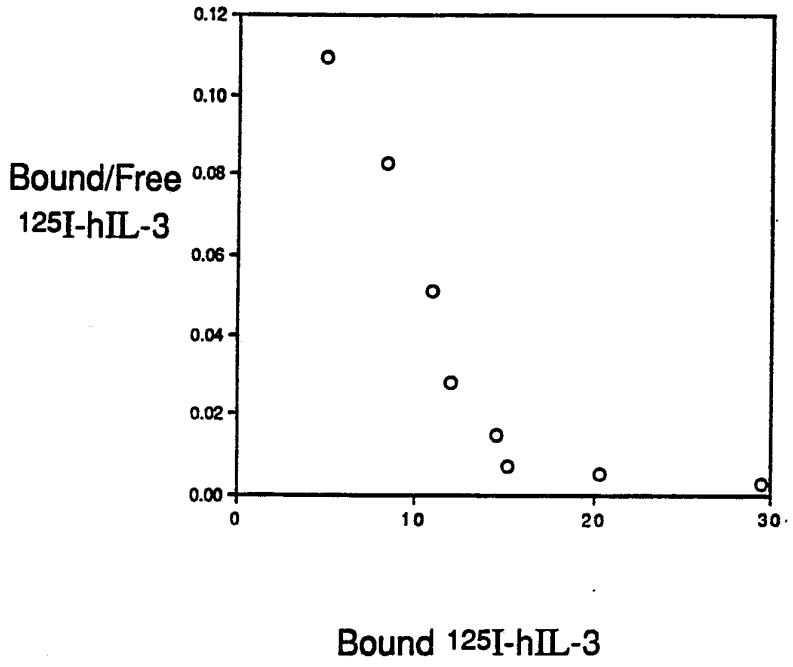
(72) Inventors; and
(75) Inventors/Applicants *(for US only) :* KITAMURA, Toshio [JP/US]; 565 Arastadero, Apt. 105, Palo Alto, CA 94306 (US). MIYAJIMA, Atsushi [JP/US]; 4159 Dake Avenue, Palo Alto, CA 94306 (US).

(74) Agents: BLASDALE, John, H., C. et al.; Schering-Plough Corporation, One Giralda Farms, Madison, NJ 07940-1000 (US).

(81) Designated States: AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH (European patent), CI (OAPI patent), CM (OAPI patent), CS, DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GA (OAPI patent), GB (European patent), GN (OAPI patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU (European patent), MC (European patent), MG, ML (OAPI patent), MR (OAPI patent), MW, NL (European patent), NO, PL, RO, RU, SD, SE (European patent), SN (OAPI patent), TD (OAPI patent), TG (OAPI patent), US.

Published
With international search report.

(54) Title: SUBUNIT OF THE HUMAN INTERLEUKIN-3 RECEPTOR



(57) Abstract

Nucleic acids encoding the α-chain of the human interleukin-3(IL-3)-receptor, as well as the α-chain itself, are provided. The α-chain may be expressed with the β-chain in cellular hosts to form compositions useful in screening agonists and antagonists of human IL-3.

FOR THE PURPOSES OF INFORMATION ONLY

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

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CS	Czechoslovakia
DE	Germany
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GN	Guinea
GR	Greece
HU	Hungary
IT	Italy
JP	Japan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
MC	Monaco
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
NL	Netherlands
NO	Norway
PL	Poland
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SN	Senegal
SU	Soviet Union
TD	Chad
TG	Togo
US	United States of America

SUBUNIT OF THE HUMAN INTERLEUKIN 3 RECEPTOR

FIELD OF THE INVENTION

The invention relates generally to the human interleukin-3-receptor (hIL-3-receptor), and more particularly, to the synthesis of a human IL-3-receptor component and to the use of the receptor component for screening agonists and antagonists of human IL-3.

BACKGROUND

Circulating blood cells are constantly replaced by newly developed cells. Replacement blood cells are formed in a process termed hematopoiesis which involves the production of at least eight mature blood cell types within two major lineages: (1) the myeloid lineage which includes red blood cells (erythrocytes), macrophages (monocytes), eosinophilic granulocytes, megakaryocytes (platelets), neutrophilic granulocytes, basophilic granulocytes (mast cells); and (2) the lymphoid lineage which includes T lymphocytes, and B lymphocytes (Burgess and Nicola, Growth Factors and Stem Cells (Academic Press, New York, 1983)). Much of the control of blood cell formation is mediated by a group of interacting glycoproteins termed colony stimulating factors (CSFs), including G-CSF, M-CSF, GM-CSF, and multi-CSF (also known as IL-3). These glycoproteins are so named because of the *in vivo* and *in vitro* assays used to detect their presence. Techniques for the clonal culture of hematopoietic cells in semisolid culture medium have been especially important in the develop-

ment of *in vitro* assays. In such cultures, individual progenitor cells (i.e., cells developmentally committed to a particular lineage, but still capable of proliferation) are able to proliferate to form a colony of maturing progeny in a manner which is believed to be essentially identical to the comparable process *in vivo*. The role of CSFs in hematopoiesis is the subject of many reviews, and is of great interest to clinical investigators who must treat blood diseases or deficiencies: e.g. Metcalf, The Hemopoietic Colony Stimulating Factors (Elsevier, New York, 1984); Clark and Kamen, Science, Vol. 236, pgs. 1229-1237 (1987); Sachs, Science, Vol. 238, pgs. 1374-1379 (1987); Dexter et al., eds., Colony Stimulating Factors (Dekker, New York, 1990); and Morstyn et al., Cancer Investigation, Vol. 7, pgs. 443-456 (1989).

The biological effects of the CSFs are mediated by specific cell surface receptors, which may consist of one or more components. Recently, several of these have been cloned and characterized, e.g. Gearing et al., EMBO J., Vol. 8, pgs. 3667-3676 (1989) (low affinity α-chain of human GM-CSF-receptor); Itoh et al., Science, Vol. 247, pgs. 324-327 (1990) (low affinity mouse IL-3-receptor); and Hayashida et al., Proc. Natl. Acad. Sci., Vol. 87, pgs. 9655-9659 (1990) (β-chain of human GM-CSF-receptor). Besides contributing to an understanding of the signal transduction process, many of these receptors will be useful screening tools for agonists and antagonists of the natural ligand. In particular, such tools may lead to the development of non-protein agonists and antagonists which would obviate many of the difficulties associated with protein therapeutics, e.g. intravenous delivery, short serum half-life, and the like.

SUMMARY OF THE INVENTION

The invention is directed to a component of the human IL-3-receptor, referred to herein as the α-chain of the human IL-3-receptor, and to compositions thereof which bind with high affinity to human IL-3. Specifically such compositions include an α-chain and β-chain of the human IL-3-receptor that can operably associate to form a high affinity receptor for human IL-3. The invention includes allelic and genetically engineered variants of the α-chain-receptor, and nucleic acids encoding the α-chain-receptor and its allelic and genetically engineered variants. Preferably, the receptor component of the invention is selected from the group of poly-

peptides of the open reading frame defined by the amino acid sequence set forth in SEQ. ID. No. 1 (immediately preceding the Claims). Although the listed sequence includes the intracellular domain of the α-chain of the receptor, it is clear that truncated forms of the sequence which retain their extracellular and transmembrane domains and their ability of operably associate with the β-chain fall within the concept of the invention.

The invention is based in part on the discovery that high affinity binding of human IL-3 involves the same β-receptor component as high affinity binding of human GM-CSF. This led to the discovery and cloning of a cDNA clone, designated pDUK-1, which expresses a protein that is capable of binding to human IL-3 with high affinity when operably associated with the β-chain of a human IL-3-receptor (or equivalently a human GM-CSF-receptor), such as encoded by the cDNA insert of pKH97 deposited with the American Type Culture Collection (ATCC) (Rockville, MD) under accession number 40847. pDUK-1 has been deposited with the ATCC under accession number 75001. The invention includes nucleic acids (i) that are effectively homologous to the cDNA insert of pDUK-1, and (ii) that encode proteins that form high affinity IL-3-receptors in association with the β-chain-receptor protein, e.g. as encoded by pKH97. As used herein, 'high affinity' in reference to IL-3-receptor binding means that IL-3 binds to the associated α- and β-chains of the receptor with a binding constant that is at least an order of magnitude less than that for binding to either component alone. More preferably, 'high affinity' means that IL-3 binds to the associated α- and β-chains of the receptor with a binding constant, K_d, less than 1 nM, and most preferably less than 200 pM.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the binding of ^{125}I-labeled human IL-3 to COS 7 cells transiently co-transfected with KH97 and pDUK-1; and

Figure 2 is a restriction map of the vector pME18.

DETAILED DESCRIPTION OF THE INVENTION

I. Obtaining and Expressing cDNAs for the Human IL-3-Receptor β-Chain

The term "effectively homologous" as used herein means that the nucleotide sequence is capable of being detected by a hybridization probe derived from a cDNA clone of the invention. The exact numerical measure of homology necessary to detect nucleic acids coding for a receptor α-chain depends on several factors including (1) the homology of the probe to coding sequences associated with the target nucleic acids that encode polypeptides other than the α-chain, (2) the stringency of the hybridization conditions, (3) the use of single-stranded or double-stranded probes, (4) the use of RNA or DNA probes, (5) the measures taken to reduce nonspecific binding of the probe, (6) the nature of the method used to label the probe, (7) the fraction of guanosine and cytidine nucleosides in the probe, (8) the distribution of mismatches between probe and target, (9) the size of the probe, and the like. Preferably, an effectively homologous nucleic acid sequence is at least seventy percent (70%) homologous to the cDNA of the invention. More preferably, an effectively homologous nucleic acid is at least ninety percent (90%) homologous to the cDNA of the invention. Most particularly, an effectively homologous nucleic acid sequence is one whose cDNA can be isolated by a probe based on the nucleic acid sequence set forth in SEQ. ID. NO. 1 using a standard hybridization protocol with no more than a few false positive signals, e.g. fewer than a hundred. There is an extensive literature that provides guidance in selecting conditions for such hybridizations: e.g. Hames et al., Nucleic Acid Hybridization: A Practical Approach (IRL Press, Washington, D.C., 1985); Gray et al., Proc. Natl. Acad. Sci., Vol. 80, pgs. 5842-5846 (1983); Kafatos et al., Nucleic Acids Research, Vol. 7, pgs. 1541-1552 (1979); and Williams, Genetic Engineering, Vol. 1, pgs. 1-59 (1981), to name a few. By way of example, the nucleic acid of SEQ. ID. NO. 1 can be used as a probe in colony hybridization assays as described by Benton and Davis, Science, Vol. 196, pg. 180 (1977). Preferably, low stringency conditions are employed for the probe employed. (The dissociation temperature depends upon the probe length.) For example, for a probe of about 20-40 bases a typical

prehybridization, hybridization, and wash protocol is as follows: (1) prehybridization: incubate nitrocellulose filters containing the denatured target DNA for 3-4 hours at 55°C in 5 x Denhardt's solution, 5 x SSPE (20 x SSPE consists of 174 g NaCl, 27.6 g $NaH_2PO_4.H_2O$, and 7.4 g EDTA in 800 ml H_2O adjusted to pH 7.4 with 10 N NaOH), 0.1% SDS, and 100 µg/ml denatured salmon sperm DNA, (2) hybridization: incubate filters in prehybridization solution plus probe at 55°C for 2 hours, (3) wash: three 15 minute washes in 300-500 ml volumes of 6x SSC and 0.1% SDS at room temperature, followed by a final 1-1.5 minute wash in 300-500 ml of 1x SSC and 0.1% SDS at 55°C. Other equivalent procedures, e.g. employing organic solvents such as formamide, are well known in the art.

Homology as the term is used herein is a measure of similarity between two nucleotide (or amino acid) sequences. Homology is expressed as the fraction or percentage of matching bases (or amino acids) after two sequences (possibly of unequal length) have been aligned. The term alignment is used in the sense defined by Sankoff and Kruskal in Chapter one of Time Warps, String Edits, and Macromolecules: The Theory and Practice of Sequence Comparison (Addison-Wesley, Reading, MA, 1983). Roughly, two sequences are aligned by maximizing the number of matching bases (or amino acids) between the two sequences with the insertion of a minimal number of "blank" or "null" bases into either sequence to bring about the maximum overlap. Algorithms are available for computing the homology of two sequences: e.g. Needleham and Wunsch, J. Mol. Biol., Vol. 48, pgs. 443-453 (1970); and Sankoff and Kruskal (cited above), pgs. 23-29. Also, commercial services and software packages are available for performing such comparisons, e.g. Intelligenetics, Inc. (Mountain View, CA); and University of Wisconsin Genetics Computer Group (Madison, Wisconsin).

Probes based on the nucleic acid sequence of the Sequence Listing can be synthesized on commercially available DNA synthesizers, e.g. Applied Biosystems model 381A, using standard techniques, e.g. Gait, Oligonucleotide Synthesis: A Practical Approach, (IRL Press, Washington D.C., 1984). It is preferable that the probe be at least 18-30 bases long. More preferably, the probe is at least 100-200 bases long. Probes of the invention can be labeled in a variety of ways standard in the art: e.g. radio-active labels, Berent et al., Biotechniques, pgs. 208-220 (May/June 1985),

Meinkoth et al., Anal. Biochem., Vol. 138, pgs. 267-284 (1984), Szostak et al., Meth. Enzymol., Vol. 68, pgs. 419-429 (1979), and the like; and non-radioactive labels, Chu et al., DNA, Vol. 4, pgs. 327-331 (1985), Jablonski et al., Nucleic Acids Research, Vol. 14, pgs. 6115-6128 (1986), and the like.

Hybridization probes can also be used to screen candidate sources of α-chain mRNA prior to library construction, e.g. by RNA blotting, Maniatis et al., Molecular Cloning: A Laboratory Manual, pgs. 202-203 (Cold Spring Harbor Laboratory, N.Y., 1982); or Hames and Higgins, eds., pgs. 139-143 in Nucleic Acids Hybridization (IRL Press, Washington, D.C., 1985). Sources of mRNA encoding the desired polypeptides include cell populations or cell lines that express, or can be induced to express, large numbers of IL-3-receptors on their surfaces, e.g. in excess of 3000-5000.

Preferably, the α- and β-chains of the IL-3-receptor are co-transfected into a mammalian expression system (i.e. host-expression-vector combination). Many reviews are available which provide guidance for making choices and/or modifications of specific mammalian expression systems: e.g. (to name a few) Kucherlapati et al., Critical Reviews in Biochemistry, Vol. 16, Issue 4, pgs. 349-379 (1984), and Banerji et al., Genetic Engineering, Vol. 5, pgs. 19-31 (1983) review methods for transfecting and transforming mammalian cells; Reznikoff and Gold, eds., Maximizing Gene Expression (Butterworths, Boston, 1986) review selected topics in gene expression in *E. coli*, yeast, and mammalian cells; and Thilly, Mammalian Cell Technology (Butterworths, Boston, 1986) reviews mammalian expression systems. Likewise, many reviews are available which describe techniques and conditions for linking and/or manipulating specific cDNAs and expression control sequences to create and/or modify expression vectors suitable for use with the present invention, e.g. Maniatis et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, N.Y., 1982); Glover, DNA Cloning: A Practical Approach, Vol. I and II (IRL Press, Oxford, 1985), and Perbal, A Practical Guide to Molecular Cloning (John Wiley & Sons, N.Y., 1984), to name only a few.

Several DNA tumor viruses have been used as vectors for mammalian hosts. Particularly important are the numerous vectors which comprise SV40 replication, transcription, and/or translation control sequences coupled to bacterial replication control sequences, e.g. the pcD vectors developed by Okayama and Berg, disclosed in Mol. Cell Biol., Vol.

2, pgs. 161-170 (1982) and in Mol. Cell Biol., Vol. 3, pgs. 280-289 (1983), both of which are incorporated herein by reference; the SV40 vectors disclosed by Hamer in Genetic Engineering, Vol. 2, pgs. 83-100 (1980), and in U.S. Patent 4,599,308, both of which are incorporated herein by reference; and the vectors additionally containing adenovirus regulatory elements, disclosed by Kaufman and Sharp in Mol. Cell Biol., Vol. 2, pgs. 1304-1319 (1982), and by Clark et al. in U.S. patent 4,675,285, both of which are incorporated herein by reference. COS7 monkey cells, described by Gluzman, Cell, Vol. 23, pgs. 175-182 (1981) and available from the ATCC (accession no. CRL 1651), are usually the preferred hosts for the above vectors. SV40-based vectors suitable for mammalian-receptor expression have been developed by Aruffo and Seed, Proc. Natl. Acad. Sci., Vol. 84, pgs. 3365-3369 and 8573-8577 (1987).

II. Binding Assays

Binding assays are accomplished by letting a ligand of unknown specificity or affinity compete with a known amount or concentration of labeled human IL-3 for receptor-binding sites of a sample of cells transfected or transformed with pDUK-1, or its equivalent. Preferably, the IL-3 is labeled by radioiodination using standard protocols, e.g. reaction with 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril described by Fraker et al., Biochem. Biophys. Res. Commun., Vol. 80, pgs. 849-857 (1978) (and available from Pierce Chemical Co. as Iodogen). Generally, the binding assay is conducted as described by Lowenthal et al., J. Immunol., Vol 140, pgs. 456-464 (1988), which is incorporated by reference. Briefly, aliquots of cells are incubated in the presence of ^{125}I-labeled human IL-3 in a final volume of 200 µl culture medium in microfuge tubes at 4°C. Cell-bound ^{125}I-labeled IL-3 was separated from non-bound ^{125}I-labeled IL-3 by centrifugation through an oil gradient (10,000 x G for 2 min). Nonspecific binding is measured in the presence of a 100-fold excess of partially purified unlabeled human IL-3.

The following Examples illustrate but do not limit the invention:

EXAMPLES

Example I. Construction of cDNA library from TF-1 cells and isolation of pDUK-1

Poly(A)+ RNA from human TF-1 cells (Kitamura et al., J. Cell Physiol., Vol. 140, pgs. 323-334 (1989)) cultured in the presence of hIL-3 (5 ng/ml) was isolated by the guanidium isothiocyanate method (Chirgwin et al., Biochemistry, Vol. 18, pgs. 5294-5299 (1978)), and was converted to double-stranded cDNA using oligo(dT) primers. After *Bst* XI linkers (containing *Xba* I sites) were ligated to both ends of the cDNAs, the cDNAs were size-fractionated through an agarose gel. cDNAs greater than 1.0 kb were digested with *Xba* I and ligated with *Xba* I-digested pME18, an SV40-based mammalian expression vector, diagrammed in Figure 2, to form a library of about 3 x 10^6 independent clones. About 3 µg of miniprep DNA from pools of 3 x 10^3 clones was co-transfected with 50 ng of pKH97 (carrying a cDNA insert encoding the β-chain of the hGM-CSF-receptor) into COS 7 cells by electroporation (0.4 gap cuvette at 300 volts and 300 µF using a Gene Pulser (BioRad, Richmond, CA)). The Cos 7 cells were incubated for 72 hours prior to screening. pDUK-1 was isolated by screening for cells capable of high affinity binding to ^{125}I-labelled hIL-3. 10 nM ^{125}I-labelled hIL-3 was added to transfected Cos 7 cells in a Chamber Slide (Labo-Tek), after which cells binding ^{125}I-labelled hIL-3 were identified by microscopic autoradiography.

Example II. Binding of hIL-3 to COS 7 cells Co-transfected with pKH97 and pDUK-1

A total of 5 µg of equal amounts of pKH97 and pDUK-1 plasmid DNA was transfected into semi-confluent COS 7 cells by the DEAE-dextran method. 72 hours after transfection, the cells were harvested and analyzed in IL-3 binding assays. Duplicates of 2x10^5 COS 7 cells in 0.1 ml of RPMI 1640 containing 10% fetal calf serum, 2 mM EDTA, 0.02% sodium azide and 20 mM Hepes (pH 7.4) were incubated for 3 hours at 4°C with various concentrations of ^{125}I-labeled human IL-3 with or without an excess amount of non-labeled human IL-3. The cell-bound radioactivity was

measured by separating the cells from free ligand by centrifugation through an oil layer, as described by Schreurs et al., Growth Factors, Vol. 2, pgs. 221-233 (1990). IL-3 was iodinated by a standard protocol, that of Chiba et al., Leukemia, Vol. 4, pgs. 22-36 (1990). Briefly, 5 µg of *E. coli*-produced human IL-3 was incubated in 30-50 µl of 50 mM sodium borate buffer (pH 8.0) with 1 mCi of the dried Bolton and Hunter reagent for 12-16 hours at 4°C. Glycine was added to 2.5 mg/ml to stop the reaction and the iodinated IL-3 was separated from the free Bolton and Hunter reagent by a PD-10 column. The iodinated human IL-3 had a specific radioactivity of (4 to 8) x 10^7 cpm/µg and was stable for about two months in Hepes-buffered Hanks's balanced salt solution containing 0.1% gelatin, 0.1% bovine serum albumin, and 0.02% sodium azide.

Figure 1 shows the receptor-binding data. Open circles correspond to COS 7 cells transfected with pKH125 and pKH97. Scatchard analysis (by the LIGAND program, De Lean et al., Mol. Pharmacol., Vol. 21, pgs. 5-16 (1982)) of the binding data indicated an equilibrium binding constant, K_d, of 100 pM.

Example III. Co-transfection of pKH97 and pDUK-1 into NIH3T3 Cells

A DNA fragment containing the neomycin-resistance gene, neo, was inserted into pKH97 downstream of the SRα promoter to form pKH97neo, and a DNA fragment containing the hygromycin-resistance gene, hyg, was inserted into pDUK-1 downstream of the SRα promoter to form pDUK-1hyg. NIH3T3 cells were stably transfected with pKH97neo and pDUK-1hyg by the calcium-phosphate procedure, described by Chen and Okayama, Mol. Cell Biol., Vol. 7, pgs. 2745-2752 (1987), which reference is incorporated by reference. Stable co-transfectants were selected by 1 mg/ml of G418 and 1 mg/ml hygromycin. Analysis of the binding of ^{125}I-labelled hIL-3 indicated a K_d of about 100 pM.

Example IV. Use of Stably Co-transfected NIH3T3 cells to screen for IL-3 Antagonists

Aliquots of NIH3T3 cells co-transfected with pKH97neo and pDUK-1hyg as described above are distributed to wells of microtiter plates in 200 µl of medium containing ^{125}I-labeled human IL-3 at concentrations

of 100 pM, 500 pM, and 1 nM. 100 µl samples of microbial supernatants free of cells are added to the transfected NIH3T3 cells at each of the different concentrations of ^{125}I-labeled IL-3. After incubation for 3 hours the NIH3T3 cells are harvested and assayed for bound radioactivity. NIH3T3 cells with low counts of bound radioactivity correspond to microbial samples containing candidate antagonists or agonists of human IL-3.

The descriptions of the foregoing embodiments of the invention have been presented for purpose of illustration and description. They are not intended to be exhaustive or to limit the invention to the precise forms disclosed, and obviously many modifications and variations are possible in light of the above teaching. The embodiments were chosen and described in order to best explain the principles of the invention to thereby enable others skilled in the art to best utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated. It is intended that the scope of the invention be defined by the claims appended hereto.

Applicants have deposited pKH97 and pDUK-1 with the American Type Culture Collection, Rockville, MD, USA (ATCC), under accession numbers 40847 and 75001, respectively. These deposits were made under conditions as provided under ATCC's agreement for Culture Deposit for Patent Purposes, which assures that the deposit will be made available to the US Commissioner of Patents and Trademarks pursuant to 35 USC 122 and 37 CFR 1.14, and will be made available to the public upon issue of a U.S. patent, which requires that the deposit be maintained. Availability of the deposited plasmids is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The Deposits have been modified to satisfy the requirements of the Budapest Treaty on the Deposit of Microorganisms.

SEQUENCE LISTING

SEQ ID NO: 1:

SEQUENCE TYPE: Nucleic acid with encoded amino acid

SEQUENCE LENGTH: 1460 bases; 359 amino acid residues

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: DNA with encoded protein

ORIGINAL SOURCE ORGANISM: human

PROPERTIES: Human IL-3-receptor

```
GCACACGGGA AGATATCAGA AACATCCTAG GATCAGGACA CCCCAGATCT TCTCAACTGG   60

AACCACGAAG GCTGTTTCTT CCACACAGCA CTTTGATCTC CATTTAAGCA GGCACCTCTG  120

TCCTGCGTTC CGGAGCTGCG TTCCCG ATG GTC CTC CTT TGG CTC ACG CTG CTC   173
                             Met Val Leu Leu Trp Leu Thr Leu Leu
                                                -15

CTG ATC GCC CTG CCC TGT CTC CTG CAA ACG AAG GAA GAT CCA AAC CCA    221
Leu Ile Ala Leu Pro Cys Leu Leu Gln Thr Lys Glu Asp Pro Asn Pro
-10                  -5                   1                   5

CCA ATC ACG AAC CTA AGG ATG AAA GCA AAG GCT CAG CAG TTG ACC TGG    269
Pro Ile Thr Asn Leu Arg Met Lys Ala Lys Ala Gln Gln Leu Thr Trp
             10                  15                  20

GAC CTT AAC AGA AAT GTG ACC GAT ATC GAG TGT GTT AAA GAT GCC GAC    317
Asp Leu Asn Arg Asn Val Thr Asp Ile Glu Cys Val Lys Asp Ala Asp
         25                  30                  35

TAT TCT ATG CCG GCA GTG AAC AAT AGC TAT TGC CAG TTT GGA GCA ATT    365
Tyr Ser Met Pro Ala Val Asn Asn Ser Tyr Cys Gln Phe Gly Ala Ile
     40                  45                  50

TCC TTA TGT GAA GTG ACC AAC TAC ACC GTC CGA GTG GCC AAC CCA CCA    413
Ser Leu Cys Glu Val Thr Asn Tyr Thr Val Arg Val Ala Asn Pro Pro
 55                  60                  65                  70

TTC TCC ACG TGG ATC CTC TTC CCT GAG AAC AGT GGG AAG CCT TGG GCA    461
Phe Ser Thr Trp Ile Leu Phe Pro Glu Asn Ser Gly Lys Pro Trp Ala
                 75                  80                  85

GGT GCG GAG AAT CTG ACC TGC TGG ATT CAT GAC GTG GAT TTC TTG AGC    509
Gly Ala Glu Asn Leu Thr Cys Trp Ile His Asp Val Asp Phe Leu Ser
             90                  95                  100
```

```
TGC AGC TGG GCG GTA GGC CCG GGG GCC CCC GCG GAC GTC CAG TAC GAC   557
Cys Ser Trp Ala Val Gly Pro Gly Ala Pro Ala Asp Val Gln Tyr Asp
        105                 110                 115

CTG TAC TTG AAC GTT GCC AAC AGG CGT CAA CAG TAC GAG TGT CTT CAC   605
Leu Tyr Leu Asn Val Ala Asn Arg Arg Gln Gln Tyr Glu Cys Leu His
    120                 125                 130

TAC AAA ACG GAT GCT CAG GGA ACA CGT ATC GGG TGT CGT TTC GAT GAC   653
Tyr Lys Thr Asp Ala Gln Gly Thr Arg Ile Gly Cys Arg Phe Asp Asp
135                 140                 145                 150

ATC TCT CGA CTC TCC AGC GGT TCT CAA AGT TCC CAC ATC CTG GTG CGG   701
Ile Ser Arg Leu Ser Ser Gly Ser Gln Ser Ser His Ile Leu Val Arg
                155                 160                 165

GGC AGG AGC GCA GCC TTC GGT ATC CCC TGC ACA GAT AAG TTT GTC GTC   749
Gly Arg Ser Ala Ala Phe Gly Ile Pro Cys Thr Asp Lys Phe Val Val
            170                 175                 180

TTT TCA CAG ATT GAG ATA TTA ACT CCA CCC AAC ATG ACT GCA AAG TGT   797
Phe Ser Gln Ile Glu Ile Leu Thr Pro Pro Asn Met Thr Ala Lys Cys
        185                 190                 195

AAT AAG ACA CAT TCC TTT ATG CAC TGG AAA ATG AGA AGT CAT TTC AAT   845
Asn Lys Thr His Ser Phe Met His Trp Lys Met Arg Ser His Phe Asn
    200                 205                 210

CGC AAA TTT CGC TAT GAG CTT CAG ATA CAA AAG AGA ATG CAG CCT GTA   893
Arg Lys Phe Arg Tyr Glu Leu Gln Ile Gln Lys Arg Met Gln Pro Val
215                 220                 225                 230

ATC ACA GAA CAG GTC AGA GAC AGA ACC TCC TTC CAG CTA CTC AAT CCT   941
Ile Thr Glu Gln Val Arg Asp Arg Thr Ser Phe Gln Leu Leu Asn Pro
                235                 240                 245

GGA ACG TAC ACA GTA CAA ATA AGA GCC CGG GAA AGA GTG TAT GAA TTC   989
Gly Thr Tyr Thr Val Gln Ile Arg Ala Arg Glu Arg Val Tyr Glu Phe
            250                 255                 260

TTG AGC GCC TGG AGC ACC CCC CAG CGC TTC GAG TGC GAC CAG GAG GAG  1037
Leu Ser Ala Trp Ser Thr Pro Gln Arg Phe Glu Cys Asp Gln Glu Glu
        265                 270                 275

GGC GCA AAC ACA CGT GCC TGG CGG ACG TCG CTG CTG ATC GCG CTG GGG  1085
Gly Ala Asn Thr Arg Ala Trp Arg Thr Ser Leu Leu Ile Ala Leu Gly
    280                 285                 290

ACG CTG CTG GCC CTG GTC TGT GTC TTC GTG ATC TGC AGA AGG TAT CTG  1133
Thr Leu Leu Ala Leu Val Cys Val Phe Val Ile Cys Arg Arg Tyr Leu
295                 300                 305                 310
```

```
GTG ATG CAG AGA CTC TTT CCC CGC ATC CCT CAC ATG AAA GAC CCC ATC  1181
Val Met Gln Arg Leu Phe Pro Arg Ile Pro His Met Lys Asp Pro Ile
                315                 320                 325

GGT GAC AGC TTC CAA AAC GAC AAG CTG GTG GTC TGG GAG GCG GGC AAA  1229
Gly Asp Ser Phe Gln Asn Asp Lys Leu Val Val Trp Glu Ala Gly Lys
            330                 335                 340
GCC GGC CTG GAG GAG TGT CTG GTG ACT GAA GTA CAG GTC GTG CAG AAA  1277
Ala Gly Leu Glu Glu Cys Leu Val Thr Glu Val Gln Val Val Gln Lys
        345                 350                 355

ACT TGAGACTGGG GTTCAGGGCT TGTGGGGGTC TGCCTCAATC TCCCTGGCCG       1330
Thr

GGCCAGGCGC CTGCACAGAC TGGCTGCTGG ACCTGCGCAC GCAGCCCAGG           1380

AATGGACATT CCTAACGGGT GGCCTGTGTA ATTTCGTTGG GCATGGGAGA           1430

TGCCGAAGCT GCCAGGAAGA AGAACAGAAC                                 1460
```

CLAIMS:

1. An α-chain of a human interleukin-3-receptor substantially free of human non-receptor proteins.

2. The protein of claim 1 wherein said α-chain is a polypeptide of the open reading frame defined by the amino acid sequence of SEQ ID NO 1.

4. A nucleic acid capable of encoding a polypeptide of an open reading frame defined by the amino acid sequence of SEQ ID NO 1.

6. A nucleic acid which is effectively homologous to the nucleotide sequence of SEQ ID NO 1 and which encodes a polypeptide capable of forming a high affinity receptor for human interleukin-3, the polypeptide forming the high affinity receptor in operable association with a β-chain of a human interleukin-3-receptor.

7. The nucleic acid of claim 6 wherein said high affinity receptor has a binding constant (K_d) with human interleukin-3 of less than 1 nM.

8. The nucleic acid of claim 7 wherein said polypeptide is operably associated with said β-chain in a mammalian expression host co-transfected with the nucleic acid and a vector carrying a gene for said α-chain.

9. A method of detecting an antagonist or agonist of human interleukin-3, the method comprising the steps of:

providing a cellular host expressing genes for the α- and β-chains of a human interleukin-3-receptor so that the α- and β-chains are operably associated in the membrane of the cellular host to form a high affinity human interleukin-3-receptor;

exposing the cellular host to a known concentration of a labelled human interleukin-3 and a sample suspected of containing an antagonist or agonist of human interleukin-3 so that the labelled human interleukin-3 can bind to the high affinity human interleukin-3-receptor;

removing the cellular host from the sample and the unbound labeled human interleukin-3; and

determining the amount of labeled human interleukin-3 that bound to the cellular host.

10. The method of claim 9 wherein said cellular host is a mammalian cell stably transformed with a first vector carrying a gene for said α-chain and a second vector carrying a gene for said β-chain.

11. The method of claim 10 wherein said first vector is pDUK-1, said second vector is pKH97 and said high affinity human interleukin-3-receptor has a binding constant with human interleukin-3 of at least 200 pM.

12. A composition of matter comprising an α-chain and a β-chain of a human interleukin-3-receptor, the α-chain and β-chain being in operable association in a non-human cellular host.

13. The composition of matter of claim 12 wherein said α-chain is encoded by the cDNA insert of pDUK-1 and said β-chain is encoded by the cDNA insert of pKH97.

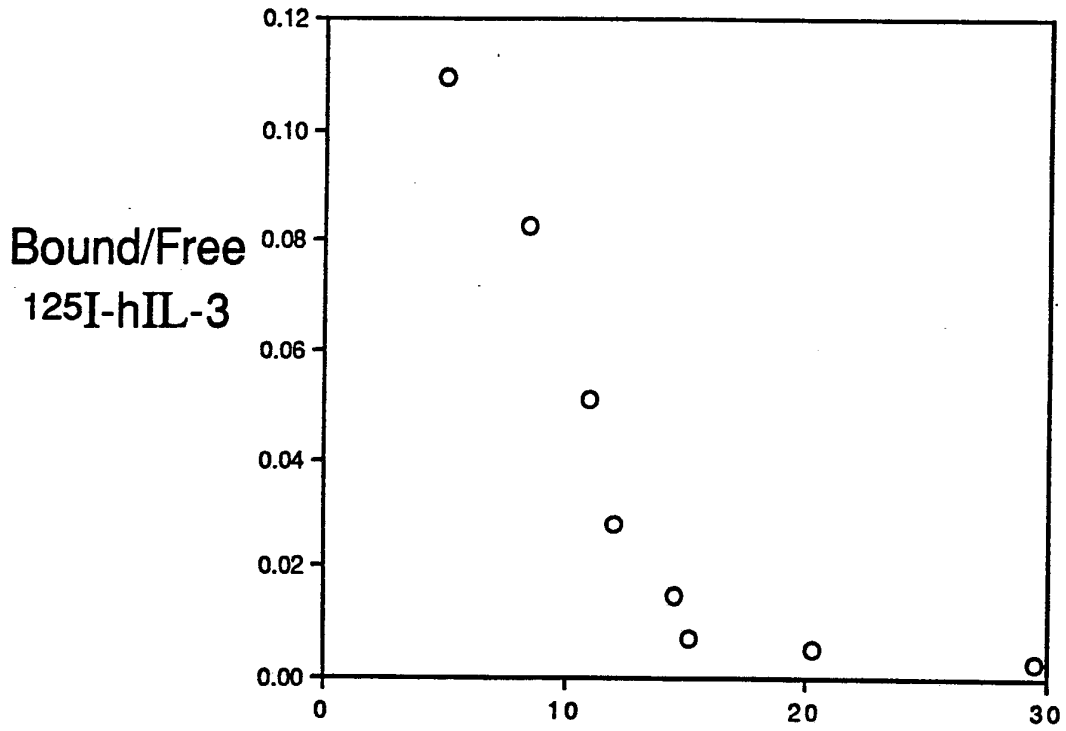

Bound/Free
125I-hIL-3
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
10
20
30
Bound 125I-hIL-3

Figure 1

2/2

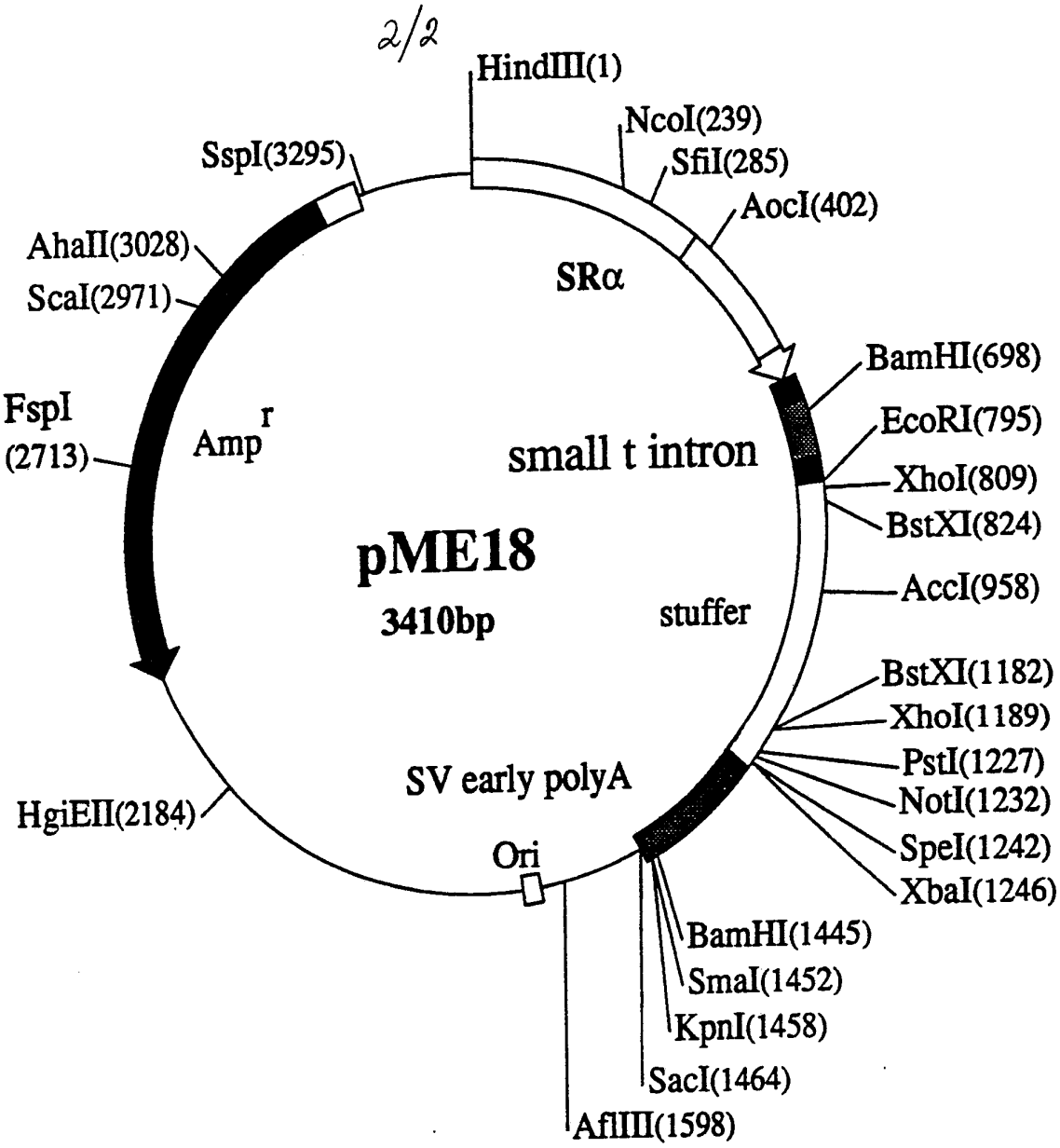

HindIII(1)
NcoI(239)
SfiI(285)
AocI(402)
SRα
BamHI(698)
EcoRI(795)
XhoI(809)
BstXI(824)
AccI(958)
BstXI(1182)
XhoI(1189)
PstI(1227)
NotI(1232)
SpeI(1242)
XbaI(1246)
BamHI(1445)
SmaI(1452)
KpnI(1458)
SacI(1464)
AflIII(1598)
Ori
SV early polyA
stuffer
small t intron
pME18
3410bp
Amp r
FspI
(2713)
HgiEII(2184)
AhaII(3028)
ScaI(2971)
SspI(3295)

Figure 2

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/03026

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

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

Int.Cl. 5 C12N15/12; C07K13/00; G01N33/58; G01N33/60
C07K15/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; C07K ; G01N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
P,X	CELL. vol. 66, no. 6, 20 September 1991, CAMBRIDGE, NA US pages 1165 - 1174; Kitamura T; Sato N; Arai K; Miyajima A: 'Expression cloning of the human IL-3 receptor cDNA reveals a shared beta subunit for the human IL-3 and GM-CSF receptors.' see the whole document --- -/--	1-13

° Special categories of cited documents :[10]

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

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

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

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

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

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

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

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

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14 JULY 1992	[illegible]
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	NAUCHE S.A.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. vol. 161, no. 1, 30 May 1989, DULUTH, MINNESOTA US pages 16 - 22; KUWAKI, TOMOAKI; KITAMURA, TOSHIO; TOJO, ARINOBU; MATSUKI, SHIGERU; TAMAI, YUKIO; MIYAZONO, KOHEI; TAKAKU, FUMIMARO: 'Characterization of human interleukin-3 receptors on a multifactor-dependent cell line.' see the whole document	1-13
A	SCIENCE. vol. 247, 19 January 1990, LANCASTER, PA US pages 324 - 327; ITOH, NAOTO; YONEHARA, SHIN; SCHREURS, JOLANDA; GORMAN, DANIEL M.; MARUYAMA, KAZUO; ISHII, AI; YAHARA, ICHIRO; ARAI, KENICHI; MIYA: 'Cloning of an interleukin-3 receptor gene: a member of a distinct receptor gene family' cited in the application see the whole document	1-13
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 87, no. 4, July 1990, WASHINGTON US pages 5459 - 5463; GORMAN, DANIEL M.; ITOH, NAOTO; KITAMURA, TOSHIO; SCHREURS, JOLANDA; YONEHARA, SHIN; YAHARA, ICHIRO; ARAI, KENICHI; MIYAJIMA, ATSU: 'Cloning and expression of a gene encoding an interleukin 3 receptor-like protein: identification of another member of the cytokine receptor gene family.' see the whole document	1-13