(54) Title: A HUMAN T-CELL RECEPTOR OF THE G-PROTEIN COUPLED RECEPTOR FAMILY

(57) Abstract

This invention relates to nucleotide sequences coding for a human T-cell receptor, to recombinant DNA comprising such nucleotide sequences, vectors comprising such recombinant DNA, and transformed cells and transgenic animals comprising such a nucleotide sequence. This invention further relates to polypeptides which function as a receptor in human T-cells, to antibodies thereto and to ligands which bind to said polypeptide. This invention also relates to an assay for the ability of an agent to act as an agonist or antagonist of said T-cell receptor and to antisense sequences to said nucleotide sequences.
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A HUMAN T-CELL RECEPTOR OF THE G-PROTEIN COUPLED RECEPTOR FAMILY

Technical Field

This invention relates to a human T-cell receptor. More specifically, this invention relates to nucleotide sequences coding for a human T-cell receptor, to recombinant DNA comprising such nucleotide sequences, vectors comprising such recombinant DNA, and transformed cells and transgenic animals comprising such a nucleotide sequence. This invention further relates to polypeptides which function as a receptor in mammalian cells, to antibodies thereto and to ligands which bind to said polypeptide. Further still, this invention relates to an assay for the ability of an agent to act as an agonist or antagonist of said T-cell receptor. Yet further still, this invention relates to antisense sequences to said nucleotide sequences.

Background Art

G proteins are well known to those skilled in the art as guanine nucleotide-binding proteins. Hence, G-protein coupled receptors are also well known in the art as receptors which are coupled to guanine nucleotide-binding protein. G proteins transmit signals from the associated receptors which are at the cell-surface to certain effector enzymes within the cell via a GTP binding and hydrolysis cycle. G-protein-coupled receptors typically have seven membrane spanning segments. Chaffin, K. E., et al., EMBO Journal 9:3821-3829 (1990) suggest that a pertussis toxin signalling process involves G, proteins and regulates thymocyte emigration.


Polypeptide analogs of an IL-8 receptor-interacting site, antibodies thereto and the use of such antibodies in the immunoassay of IL-8 and to treat inflammation are disclosed in WO9204372, published March 19, 1992. The human complement receptor type 1 gene, the encoded protein and fragments thereof, and uses of such proteins and fragments for treatment of immune disorders, myocardial infarct, damage due to inflammation and, in combination with a thrombolytic, thrombosis are disclosed in WO9105047, published April 18, 1991. The preparation and use of a C5a receptor protein is disclosed in EP377489, published July 11, 1990.

Disclosure of the Invention

This invention provides a T-cell receptor and nucleotide sequences encoding the receptor. The nucleotide sequences of this invention consist essentially of the nucleotide sequences shown hereinbelow as SEQUENCE I.D. NO: 1 and SEQUENCE I.D. NO: 5. Notwithstanding the nucleotide sequence presented herein as SEQUENCE I.D. NO: 1, this invention further provides functional equivalents of that sequence together with functional fragments thereof and functional equivalents of the functional fragments. Included therein is the nucleotide sequence of SEQUENCE I.D. NO: 5, as well as functional equivalents thereof together with functional fragments thereof and functional equivalents of such functional fragments. Preferred are the nucleotide sequences of SEQUENCE I.D. NO: 1 and SEQUENCE I.D. NO: 5.

This invention further provides recombinant DNA comprising a nucleotide sequence of SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5, a functional equivalent of SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5, a functional fragment of SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5, or a functional equivalent of a functional fragment of SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5. Preferred is such recombinant DNA wherein the nucleotide sequence is functionally linked to a promoter.

This invention still further provides vectors comprising recombinant DNA as hereinabove described.

Also provided by this invention are transformed or transfected host cells comprising a nucleotide sequence of SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5, a functional equivalent of SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5, a
functional fragment of SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5, or a functional equivalent of a functional fragment of SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5. The transformed or transfected host cells of this invention are all cells suitable for the replication and/or expression of the nucleotide sequences hereinabove described. By way of example and not of limitation such cells include *Escherichia coli*, *Saccharomyces cerevisiae*, *Yarrowia lipolytica* and mammalian cells.

Yet further still, this invention provides transgenic non-human animals comprising a nucleotide sequence of SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5, a functional equivalent of SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5, a functional fragment of SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5 or a functional equivalent of a functional fragment of SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5.

This invention further provides antisense sequences to SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5 as well functional equivalents thereof, functional fragments of SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5 and functional equivalents of such functional fragments. The antisense sequences of this invention include all such sequences which are the antisense of the expression sequences of the sequences described above as well as all functional equivalents, functional fragments and functional equivalents of functional fragments of said antisense sequences. The term expression sequence when used in the singular or plural includes, but is not limited to the nucleotide sequences which code for the translational controls and/or the structural sequence. The antisense sequences of this invention are useful to down-regulate the expression of polypeptide SEQUENCE I.D. NO: 2, as well as functional equivalents and fragments thereof as defined below, in cells and, thus, could find usefulness in treating graft rejection and/or inflammatory conditions in an animal, including a human being. Also included in this invention are modified oligonucleotides which bind to said antisense sequences as well as compositions comprising such modified oligonucleotides and antisense sequences.

This invention also provides a substantially pure polypeptide of SEQUENCE I.D. NO: 2. Also, provided by this invention are functional equivalents of SEQUENCE I.D. NO: 2, functional fragments of SEQUENCE I.D. NO: 2 and functional equivalents of functional fragments of SEQUENCE I.D. NO: 2. The polypeptide of SEQUENCE I.D. NO: 2 is encoded by the nucleotide sequence of SEQUENCE I.D. NO: 5 as well as SEQUENCE I.D. NO: 1 and is a putative human T-cell G-protein coupled receptor. The
polypeptides of this invention as hereinabove described are useful for preparing antibodies thereto. Further, the polypeptides are useful in assay procedures such as those described hereinbelow for agonists or antagonists of a T-cell receptor. Still further, the polypeptides of this invention comprising a binding site are useful in the preparation of soluble polypeptides or fragments thereof which inhibit receptor function by competing for binding to the ligand. The polypeptides of this invention are also useful in the isolation of a ligand of the receptor formed by the polypeptide of SEQUENCE I.D. NO: 2 as well as identification of clones expressing a natural ligand thereof from within an expression library.

This invention also provides methods to assay an agent for the ability thereof to act as a ligand or an agonist of a T-cell receptor comprising a polypeptide of SEQUENCE I.D. NO: 2, functional equivalents thereof, functional fragments of SEQUENCE I.D. NO: 2 or functional equivalents of functional fragments of SEQUENCE I.D. NO: 2; methods to assay an agent for the ability thereof to act as a selective antagonist of such a T-cell receptor; and methods to assay an agent for the ability thereof to act as an antagonist of such T-cell receptors. The methods are set forth hereinbelow.

Also provided by this invention are the antibodies herein described which are useful for identifying cells expressing the receptor formed by the polypeptide of SEQUENCE I.D. NO: 2 and could find usefulness in treating graft rejection and/or inflammatory conditions in an animal, including a human being. Preferably the antibodies are monoclonal antibodies.

Still further, this invention provides a ligand to the receptor which ligand is useful in a receptor binding assay to identify a receptor antagonist or agonist.

As used throughout this Specification and the appendant claims, "functional fragment" means a fragment of the sequence to which the phrase refers which fragment has a function which is at least part of the overall function of the complete sequence.

As used throughout this Specification and the appendant claims, "functional equivalent" when used in the singular or plural means a sequence having the same, or substantially the same function as the sequence to which the phrase refers.

As used throughout this Specification and the appendant claims, "recombinant DNA" means DNA which comprises a nucleotide sequence according to this invention regardless of the nature of the DNA. Thus, by way of example and not of limitation, recombinant DNA includes linear DNA fragments as well as chromosomal DNA wherein
a nucleotide sequence according to this invention has been inserted as well as
derivatives by replication thereof.

Description of the Drawing

Figure 1 is a map of plasmid pCR11/Da6 wherein pBR322 ori represents the
origin of replication from plasmid pBR322, Ampicillin represents the coding sequence
for ampicillin resistance, Kanamycin represents the coding sequence for Kanamycin
resistance, f1 ori represents the origin of replication from phage f1, Lac Z represents
the partial coding regions for β-galactosidase, MCS represents a multiple cloning site,
DA6 represents the sequence containing SEQUENCE I.D. NO: 1 as described herein,
Hpall represent the restriction site locations for the enzyme Hpall, ATG represents the
location of the start codon for the coding sequence of Da6 (i.e., SEQUENCE I.D. NO:
5) and STOP represents the location of the stop codon at the 3' terminus of the coding
sequence of Da6 (i.e., SEQUENCE I.D. NO: 5).

Detailed Description

1. PREPARATION AND IDENTIFICATION OF CLONE Da6

Employing the guanidium isothiocyanate method described by Chomczynski,
P., et al., Analytical Biochemistry 162: 156-159 (1987), mRNA was isolated from fresh
human peripheral blood T cells. According to methods well known to those skilled in
the art, and as described by Molecular Cloning, Second Edition, Part 2, Sambrook, J.,
8.60-8.63, first strand cDNA was generated from the mRNA using reverse transcriptase.
Two primers were commercially prepared by Genosys Corp, Houston, TX. One primer
was a degenerate oligonucleotide corresponding to a consensus sequence obtained
by comparison of the M2 transmembrane domain of the human interleukin 8 ([IL-8,
Holmes, W. E., et al., Science 253:1278-1280 (1991)], F-met-Leu-Phe [FMLP, Boulay,
Biological Chemistry 265:20455-20462 (1990]), and glucocorticoid-induced receptor
[MUSGCP, Harrigan, M.T., et al., Molecular Endocrinology 5:1331-1338 (1991)]. The
M2 degenerate primer was of the sequence ATAGCTTANNTNGCNNTNGCNGAC
(SEQUENCE I.D. NO: 3). The second primer was a degenerate oligonucleotide
corresponding to a consensus sequence obtained by comparison of the M6
transmembrane domain of an IL-8, hFMLP, hC5a, hNKA and MUSGCP receptor. The
M6 degenerate primer (reverse complement) was of the sequence 
TCGAATTCNANNTNNTANGGNNNCCA (SEQUENCE I.D. NO: 4).

Using the M2 and M6 degenerate primers of SEQUENCE I.D. NO: 3 and
SEQUENCE I.D. NO: 4, the cDNA prepared as described above was amplified using a
modified PCR amplification protocol whereby the DNA was amplified 30 cycles with a
30-second denaturization temperature of 94°C and a 45-second elongation temperature
of 72°C. The annealing temperature was 37°C for the first cycle and the temperature
was increased 0.5°C per cycle thereafter. The resulting amplified sequences where
cloned into plasmid pBSK (Stratogene, LaJolla, CA) as a restriction fragment
(EcoRI/HindIII). Resulting clones were sequenced using automated fluorescent
sequencing on Applied Biosystems 373A Automated DNA sequences (Applied
Biosystems Inc. Foster City, CA).

A partial Daa6 clone was identified by comparison of the translated sequence of
the clones to the PIR protein database using BLAST sequence comparison software
[National Center for Biotechnology Information, National Library of Medicine, NIH,
Bethesda, MD; Altschul, S.F., et al., Journal of Molecular Biology 215:403-410 (1990)].
The partial Daa6 clone was then used to isolate the complete Daa6 clone by hybridization
to a human thymus A-gt-11 cDNA library (Clontech, Palo Alto, CA). Initially, about
500,000 plaques, immobilized on nitrocellulose filters, were hybridized to the partial Daa6
clone in 5X Denhardt's solution, 5X SSPE, 50% formamide, 100ug/ml ssDNA, 0.1%
SDS, and 10% Dextran sulfate at 42°C for 16 hours. The filters then were washed in
0.16X SSC/1.0% SDS at 50°C for 30 minutes. Then, the filters were autoradiographed
to X-ray film, with intensifying screens, for 12 hours at -70°C. Resulting positive clones
were subjected to two more rounds of plaque screening as described above using the
partial Daa6 cDNA to purify the full length Daa6 clone. A resulting positive clone was
amplified and its DNA was recovered as outlined in Molecular Cloning, Second Edition,
Part 1, Sambrook, et al., Op. cit., pp. 2.69-2.71. Purified DNA was then subjected to
PCR (Hoffman LaRoche, Nutley, NJ) using commercially available DNA primers that
flank the cloning region of Agt11 (Clonetech, Palo Alto, CA). The amplified sequences
were then cloned into pCRII - 'TA Cloning System' (Invitrogen, San Diego, CA) using the
ligation conditions supplied by the manufacturer. The full length Daa6 clone was verified
by fluorescent automated sequencing (Applied Biosystems Inc., Foster City, CA).
Clone Daa6 contains a unique sequence based on searches of both the PIR and
Genbank sequence databases (Intelligenetics, Inc., Mountain View, CA). Due to certain
homology between Da6 and the platelet activating factor and IL-8 receptors, it was concluded that Da6 encodes a novel putative G-protein coupled receptor. A map of plasmid pCRII/Da6 is presented in Figure 1.

Using Da6 as a probe, it was shown by Northern analysis (Molecular Cloning, Second Edition, Part 1, Sambrook, J., et al., Op. cit., pp 7.37-7.52) that the receptor encoded thereby is preferentially expressed in T cells. The nucleotide sequence of the receptor of clone Da6, including its associated 5' and 3' nucleotide sequences, is shown below as SEQUENCE I.D. NO: 1.

Clone Da6 which comprises SEQUENCE I.D. NO: 1 in pCRII (Invitrogen, San Diego, CA) has been deposited under the terms of the Budapest Treaty in the American Type Culture Collection, Rockville, MD, a recognized depository affording permanence of the deposits and ready accessibility thereto by the public if a patent is granted on this application. Clone Da6 comprises Escherichia coli transformed with and containing plasmid pCRII/Da6. The HpaII fragment of pCRII/Da6 contains 999bp of Da6 coding sequence (SEQUENCE I.D. NO: 5), 60 bp of Da6 5' flanking sequence (SEQUENCE I.D. NO: 6), and 554 bp of Da6 3' flanking sequence (SEQUENCE I.D. NO: 7). The deposit is made available during the pendency of this application to one determined by the Commissioner of the United States Patent and Trademark Office to be entitled thereto under 37 C.F.R. 1.14 and 35 U.S.C. 122, and in accordance with foreign patent laws in countries wherein counterparts of this application, or its progeny, are filed. All restrictions on the availability to the public of the microorganism deposited will be irrevocably removed upon granting of the patent. Clone Da6 has been assigned deposit number ATCC 69248.

2. EXPRESSION OF SEQUENCE I.D. NO: 1 IN MAMMALIAN CELLS

The full length nucleotide sequence of SEQUENCE I.D. NO: 1 is excised from pCRII/Da6 using restriction enzyme HpaII and is blunt end cloned into the Eco RV restriction sites of vector pcDNA/Neo (Invitrogen, San Diego, CA). For purposes of subsequent antibody recognition, a synthetic flag sequence, CCAGCAAGCCATGACTACAAGGACGACGACGACAA (SEQUENCE I.D. NO: 8), is inserted at the 5' end of SEQUENCE I.D. NO: 1 in vector pcDNA/Neo by ligation of annealed complementary oligonucleotides containing this sequence in translational frame upstream of the Da6 coding sequence (Harlow, E., et al., Antibodies, 1988, Cold Spring Harbor Laboratory, New York). The vector is then amplified in transformed, competent E. coli cells and purified from those cells using CsCl gradient purification

3. PRODUCTION OF ANTIBODIES TO SEQUENCE I.D. NO: 2

Polypeptides corresponding to unique sequences formed within putative extracellular domains of SEQUENCE I.D. NO: 2 [i.e., WFLMYPPRFHDCKQKYDLYI (SEQUENCE I.D. NO: 9) and PLLRTSDDTSGNRTKCFVDLPTRNV (SEQUENCE I.D. NO: 10)] are synthesized and conjugated to an antigen such as key hole limpet hemocyanin (KLH) or bovine serum albumin (BSA). The conjugates are resuspended in an adjuvant and used to immunize and boost animals and sera is drawn after each boost. The sera is tested using an ELISA for the ability to bind the conjugated peptide but not a control peptide. Sera that reacts to the conjugated peptide is then tested for the ability to bind to the surface of SEQUENCE I.D. NO: 2 expressing cells using FACS, ELISA, immunoprecipitation, or Western blotting or immunocytohistochemistry. Alternatively, fusion proteins are prepared using polypeptide sequences from SEQUENCE I.D. NO: 2 and used to immunize animals to produce antisera. To produce such fusion proteins, nucleotide sequences from SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5 can be ligated to lacZ DNA or other DNA and expressed in *E. coli*. The fusion protein is then purified and used to immunize animals as described above.

Alternatively and preferably, monoclonal antibodies to the appropriate extracellular polypeptide sequence are prepared according to standard techniques
(Harlow, E., et al., Antibodies, 1988, pp. 55-240, Cold Spring Harbor Laboratory, NY). Polypeptides or fusion proteins containing peptide sequences from SEQUENCE I.D. NO: 2 are used to immunize mice. An appropriate amount of antigen peptides or fusion proteins (5-50 µg) are mixed with complete adjuvant and injected into the intraperitoneal cavity of mice. Alternatively, the antigen may be delivered by subcutaneous injection or by injection directly into lymphoid organs. Subsequent injections are made using incomplete Freund's adjuvant. The sera is tested using an ELISA for the ability to bind to the appropriate peptide antigen but not a control peptide antigen. Sera that reacts to the appropriate antigen is then tested for the ability to bind to the surface of SEQUENCE I.D. NO: 2 expressing cells using FACS, ELISA, immunoprecipitation or immunocytochemistry. Animals that produce antisera that reacts with the appropriate antigen are used to make hybridomas producing monoclonal antibodies. Animals are boosted with the appropriate antigen 3-5 days prior to fusion. Splenocytes are obtained from the mice and fused to suitable myeloma cells using polyethylene glycol (Harlow, E., et al., Antibodies, Ibid.). The hybridomas are selected for using hypoxanthine, aminoptesin and thymidine Selection (HAT) medium or other suitable selection medium. Hybridoma pools are tested for antibody production using ELISA assay. Positive pools are cloned and individual clones are similarly tested for production of appropriate antibodies. Positive clones are expanded and grown to produce supernatants containing the monoclonal antibody to SEQUENCE I.D. NO: 2. The monoclonal antibodies may be purified on protein A columns (Harlow, E., et al., Antibodies, Op. cit.) or by other methods known to those skilled in the art of protein purification.

4. DETECTION OF Da6 FUNCTION BY CALCIUM FLUX ASSAY

A. Fura-2 assay. SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5, or a functional equivalent thereof, a functional fragment thereof or a functional equivalent of a functional fragment thereof, transfected cells are incubated with fura-2 acetoxyethyl ester (2.5µM) in calcium/magnesium-free PBS for 30 min. at 37°C. Then, the cells are washed, resuspended in calcium/magnesium-free PBS containing 10mM HEPES pH7.4, 0.25% BSA and 10mM glucose. The cells are dispersed into quartz cuvettes and the external calcium concentration is adjusted to 1mM with CaCl₂. Receptor ligand is added at an effective concentration and fluorescence is monitored using a fluorescence spectrophotometer at excitation wavelengths of 340 nm and 380 nm and emission
wavelength of 510nm. Calcium levels are measured using the ratio of the resulting fluorescence readings.

B. **Flow cytometry.** According to the method described by Holmes, W. E. et al., Science 253: 1278-1280 (1991), cells transfected with SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5, or a functional equivalent thereof, a functional fragment thereof or a functional equivalent of a functional fragment thereof, are incubated with indo-1 acetoxyethyl ester (2μM) in RPMI for 30 min. at 37°C. The intracellular calcium is then measured with a flow cytometer from the ratio of fluorescence at wavelengths of 405 nm and 525 nm.

5. DETECTION OF Da6 FUNCTION BY REPORTER ASSAY

Transfection competent cells are co-transfected using electroporation or other transfection protocols (Molecular Cloning, Second Edition, Part 3, Sambrook, J., et al., Op. cit., pp. 16.30-16.55) with a vector capable of expressing SEQUENCE I.D. NO: 2, or a functional equivalent thereof, a functional fragment thereof or a functional equivalent of a functional fragment thereof, in said cells and a vector encoding luciferase under control of a G-protein sensitive promoter (e.g., minimal multimerized CRE, NFκB or minimal multimerized AP-1 promoter driving luciferase as CAT). Minimal multimerized CRE, NFκB or AP-1 promoter luciferase/CAT plasmids are derived by cloning a ligated double strand sequence containing multiple copies of the CRE, NFκB or AP-1 consensus sequence binding sites upstream of a minimal thymidine kinase or IL-2 promoter. The co-transfected cells are stimulated with an effective amount of receptor ligand and functioning of the SEQUENCE I.D. NO: 2 receptor is measured by the amount of luciferase or CAT activity produced by the cell as described in Molecular Cloning, Second Edition, Part 3, Sambrook, J. et al., Op. cit., pp. 16.60-16.65, and by DeWet, J.R., et al., Molecular and Cellular Biology 7:725-737 (1987).

6. PROCESS FOR IDENTIFICATION OF NATURAL LIGANDS AND AGONISTS OF SEQUENCE I.D. NO: 2

Employing transfected cells according to the Fura-2, flow cytometry or reporter assay described hereinabové and incubating the cells in the presence of a chemical entity in an appropriate solubilized form, a natural product, a purified factor or a crude cellular extract at varying concentrations and assaying for the effect thereof upon said cells when compared to non-transfected cells enables selection of agonists and natural ligands of SEQUENCE I.D. NO: 2, or a functional equivalent thereof, a functional fragment thereof or a functional equivalent of a functional fragment thereof, as those
entities which stimulate calcium flux (Fura-2 or flow cytometry assay) or increase luciferase/CAT activity (reporter assay).

7. IDENTIFICATION OF SELECTIVE SEQUENCE I.D. NO: 2 ANTAGONISTS

Cells, stably transfected with and capable of expressing SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5, or a functional equivalent thereof, a functional fragment thereof or a functional equivalent of a functional fragment thereof, are incubated in the presence of a defined concentration of a chemical entity prior to the addition thereto of a natural ligand or agonist of SEQUENCE I.D. NO: 2, or a functional equivalent thereof, a functional fragment thereof or a functional equivalent of a functional fragment thereof. In order to assay for selectivity of the antagonist, a known G-protein coupled receptor containing cell line (i.e., an IL-8R expressing control cell line) is also preincubated with the chemical entity prior to addition of a natural ligand (e.g. IL-8) or agonist thereof. In both cases, the effect of the entity on either calcium flux or reporter activity is determined. Those chemical entities which inhibit or block ligand or agonist activation of calcium flux in the stably transfected cells but not in the control cell line are antagonists selective for SEQUENCE I.D. NO: 2, or a functional equivalent thereof, a functional fragment thereof or a functional equivalent of a functional fragment thereof.

Alternatively, a receptor binding assay as described in any one of Kunz, D., et al., J. Biol. Chem. 267: 9101-9106 (1992), Nourshargh, S., et al., J. Immunol. 148: 106-111 (1992), Holmes, W.E., et al., Science 253: 1278-1280 (1991) or Gerard, N.P., et al., Biochemistry 29: 9274-9281 (1990) employing a labeled ligand of SEQUENCE I.D. NO: 2 can be employed. Transfected cells capable of expressing SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5, or a functional equivalent thereof, a functional fragment thereof or a functional equivalent of a functional fragment thereof, are pre-incubated with varying concentrations of a chemical entity for a pre-determined period of time under appropriate binding conditions followed by the addition thereto of a labeled ligand that binds to SEQUENCE I.D. NO: 2, or a functional equivalent thereof, a functional fragment thereof or a functional equivalent of a functional fragment thereof. As a control, the binding assay is also performed using the same chemical entity but with a control receptor (e.g. IL-8R) transfected into the same host cell line as was used to obtain the SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5, or a functional equivalent thereof, a functional fragment thereof or a functional equivalent of a functional fragment thereof, transfected cells and with a labeled ligand of the control receptor. A chemical entity which blocks or inhibits the binding of the labeled ligand to the SEQUENCE I.D. NO:
1 or SEQUENCE I.D. NO: 5, or a functional equivalent thereof, a functional fragment thereof or a functional equivalent of a functional fragment thereof, transfected cells but not control cells is an antagonist selective for SEQUENCE I.D. NO: 2.

8. TRANSGENIC ANIMALS COMPRISING

SEQUENCE I.D. NO: 1 OR SEQUENCE I.D. NO: 5

A transgenic vector is constructed comprising SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5, or a functional equivalent thereof, a functional fragment thereof or a functional equivalent of a functional fragment thereof, together with transcriptional and processing elements to allow expression in the tissue or tissues of choice in the transgenic animal. Expression may be directed to T lymphocytes using vectors described [Cooke, M.P., et al., Cell 65:281-291 (1991)]. Once constructed and amplified by methods well known to those skilled in art, approximately 200 copies of linear vector DNA are microinjected into the male pronucleus of the fertilized egg of the animal to be made transgenic according to the methods described by Hanahan, D., Science 246: 1265-1275 (1989) and Brinster, R. L., et al., The Harvey Lecture Series 80: 1-38 (1986). The injected eggs are then implanted into the oviduct of pregnant foster mothers and the offspring are tested for transgene integration using dot blot analysis, Southern analysis or PCR analysis as described in Molecular Cloning, Second Edition, Part 2, Sambrook, J., et al., Op. cit., pp. 9.4-9.58 and 14.5-14.21. Tissue from animals positive for transgene integration is tested for transgene expression using Northern analysis or PCR analysis for detection of transgene mRNA levels. Transgene positive animals that express the transgene are propagated and the offspring thereof are tested for transgene presence and function to verify establishment of a transgenic animal line. Such transgenic animals are useful for the generation of animal disease models resulting from overexpression of receptors from SEQUENCE I.D. NO: 2. These animals are also useful for testing the effects of antagonists, agonists or natural ligands on receptor function in vivo.

9. ANTISENSE SEQUENCES

A synthetic antisense DNA sequence of approximately 15 to 30 base pairs in length and complementary to a portion of an expression sequence of SEQUENCE I.D. NO: 1, SEQUENCE I.D. NO: 5, a functional equivalent thereof, a functional fragment of SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5, or a functional equivalent of such a functional fragment. The antisense sequence is prepared by standard methods well known to those skilled in the art such as by the use of a DNA synthesizer (Applied
Biosystems, Inc., Mountain View, CA). The antisense sequence is then administered to a T-cell or animal at an amount effective to inhibit expression of SEQUENCE I.D. NO: 2, a functional equivalent thereof, a functional fragment of SEQUENCE I.D. NO: 2 or a functional equivalent of such a functional fragment. Further, modified oligonucleotides which bind to the antisense sequences and result in increased cellular uptake, in vivo absorption, bioavailability and/or protection from nucleases can be prepared and employed in compositions with such antisense sequences according to methods described by Cohen, J.S., Pharmac. Ther. 52: 211-225 (1991).
SEQUENCE LISTING

(1) GENERAL INFORMATION:
   (i) APPLICANT: Hanke, Jeffrey H.
        Ogborne, Kevin T.
        Pfizer Inc., (non-U.S.)
   (ii) TITLE OF INVENTION: HUMAN RECEPTOR
   (iii) NUMBER OF SEQUENCES: 10
   (iv) CORRESPONDENCE ADDRESS:
        (A) ADDRESSEE: Gregg C. Benson, Pfizer Inc
        (B) STREET: Eastern Point Road
        (C) CITY: Groton
        (D) STATE: Connecticut
        (E) COUNTRY: U.S.A.
        (F) ZIP: 06340
   (v) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
   (vi) CURRENT APPLICATION DATA:
        (A) APPLICATION NUMBER:
        (B) FILING DATE:
        (C) CLASSIFICATION:
   (vii) ATTORNEY/AGENT INFORMATION:
        (A) NAME: Benson, Gregg C.
        (B) REGISTRATION NUMBER: 30,997
        (C) REFERENCE/DOCKET NUMBER: PCS348AGCB
   (ix) TELECOMMUNICATION INFORMATION:
        (A) TELEPHONE: (203) 441-4901
        (B) TELEFAX: (203) 441-5221

(2) INFORMATION FOR SEQ ID NO:1:
   (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 1613 base pairs
        (B) TYPE: nucleic acid
   (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear
   (ii) MOLECULE TYPE: cDNA
   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
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       ATGCCGTGCA ATACACGCT GACGAGGCA GATGGACAC ATACAGATT TGGATCTTT
       ATTTATGCAG TGACATACAG TGCGATCTT TGCCAGGTC TCATAGGGA TATATAGGCC
       CTGTGGGTAT TCTATGTTA TATGAAAGAA ACCAACCAG CTGTGATATT TATGATAAAAC

       60                                          120
       180                                          240
-15-
TTAGCCATTG CTGACTTACT ACAAGTTCTT TCCCTGCCAC TGAGGATCTT CTACTACTTG
AATCATGACT GCCATTTGG GCCGTCTTC TGCGATTCG TTCTCTACTG GAATATGTC
ACGATGTA TGAGCCTCTA TTCTGTTGTC TGCGACGTA TGGGGGCAT TTGCTTTTCC
AGTACCCT TTGGCTTCCA TGACTGCAA CAGAAATATG ACCTGTACAT CAGCATTTGCT
5  GCGTGCCTGAA TCTATGGGTCT GCTCGTTGTA TCTTCCTCAG GCTCTGAGAC CTGGTAGATG
ACCCCTGCCA ATAGGAGCAG AAAGTGGTTG GATCCTCTCA CAGGAAATGT CAACCCTGCC
CAGTCCGTTG TAATGAGTAC CATTGGCAAG TTGATGGGT TGGAATCTCC CGCTTCTGATT
GTCTATATT GTACCTAGGA GACGTTTAAAA TCACTGCAAG ATATAATCTC CATGCGCCCA
GATCTGGGAG AGAARAGAGAA AGCGCTTGAAG ATGATTCTAA CCCTGCAGCG GTATTCCCTA
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AGCCGCTTTT CAGACAGAGA TTTGCAATGAC AGCACCCAC TCCCAGCAAAA TATCCTTTTG
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15 ATGTGACCTG AAATCTAGAC ATATCGTGCA TACCAAGGCC ACCCCAGAAA
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CTCCAAAGGC AGCGTCTTAT TGGACGGAT CGAATCTCGA TTTAGATGTC TGACATGCTCC
AGTAGTAAAT TTTTCTCAA GTCCCTAAAT CTTAATAAT CAAATTCGG TGACATCTCA
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20 TTATTAGCTT GGAGTCACCA TAAACATTATA GGTGTTGTCG AACAGATATCT GAGTCTTTAT
GTCGACAGGA AATGTAGCTG TCTTTTTATTA TTTAGATCTCA CAGTTTTACAT GCCATTTTCA
TATGTTTGT TATTTTTTAG TGGGATAGAT GATATATAC CCCCTGGTAA AAAAGTTAAA
ACGTAAGACTC ATTTTTATCT TCTAGGTGGT ATTATCTCTC AGAGTTTGAAC CGG
30 (2) INFORMATION FOR SEQ ID NO:2:
(ii) MOLECULE TYPE: protein
35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
Met Pro Ala Asn Tyr Thr Cys Thr Arg Pro Asp Gly Asp Asn Thr Asp
1    5   10   15
Phe Arg Tyr Phe Ile Tyr Ala Val Thr Tyr Thr Val Ile Leu Val Pro
20   25   30
Gly Leu Ile Gly Asn Ile Leu Ala Leu Trp Val Phe Tyr Gly Tyr Met
35  40   45
Lys Glu Thr Lys Arg Ala Val Ile Phe Met Ile Asn Leu Ala Ile Ala
50  55   60
Asp Leu Leu Glu Val Leu Ser Leu Pro Leu Arg Ile Phe Tyr Tyr Leu
65  70   75   80
Asn His Asp Trp Pro Phe Gly Pro Gly Leu Cys Met Phe Cys Phe Tyr
85  90   95
Leu Lys Tyr Val Asn Met Tyr Ala Ser Ile Tyr Phe Tyr Val Val Cys Ile
-16-  

100 105 110  
Ser Val Arg Arg Phe Trp Phe Leu Met Tyr Pro Phe Arg Phe His Asp  
115 120 125  
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145 150 155 160  
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165 170 175  
Val Asn Leu Ala Gln Ser Val Val Met Met Thr Ile Gly Glu Leu Ile  
180 185 190  
Gly Phe Val Thr Pro Leu Leu Ile Val Leu Tyr Cys Thr Trp Lys Thr  
195 200 205  
Val Leu Ser Leu Gln Asp Lys Tyr Pro Met Ala Gln Asp Leu Gly Glu  
210 215 220  
Lys Gln Lys Ala Leu Lys Met Ile Leu Thr Cys Ala Gly Val Phe Leu  
225 230 235 240  
Ile Cys Phe Ala Pro Tyr His Phe Ser Phe Pro Leu Asp Phe Leu Val  
245 250 255  
Lys Ser Asn Glu Ile Lys Ser Cys Leu Ala Arg Arg Val Ile Leu Ile  
260 265 270  
Phe His Ser Val Ala Leu Cys Leu Ala Ser Leu Asn Ser Cys Leu Asp  
275 280 285  
Pro Val Ile Tyr Tyr Phe Ser Thr Asn Glu Phe Arg Arg Arg Leu Ser  
290 295 300  
Arg Gln Asp Leu His Asp Ser Ile Gln Leu His Ala Lys Ser Phe Val  
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325 330

(2) INFORMATION FOR SEQ ID NO:3:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 26 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  

(ii) MOLECULE TYPE: cDNA  

(ix) FEATURE:  
(A) NAME/KEY: modified_base  
(B) LOCATION: 11  
(D) OTHER INFORMATION: /mod_base= OTHER  

/note= "c or t"  

(ix) FEATURE:  
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(B) LOCATION: 12
(D) OTHER INFORMATION: /mod_base= OTHER
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  (A) NAME/KEY: modified_base
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  (D) OTHER INFORMATION: /mod_base= OTHER
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  (D) OTHER INFORMATION: /mod_base= OTHER
      /note= "g or c"

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  (B) LOCATION: 18
  (D) OTHER INFORMATION: /mod_base= OTHER
      /note= "g, c or t"

(ix) FEATURE:
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  (A) NAME/KEY: modified_base
  (B) LOCATION: 20
  (D) OTHER INFORMATION: /mod_base= OTHER
      /note= "g or a"

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  (A) NAME/KEY: modified_base
  (B) LOCATION: 23
  (D) OTHER INFORMATION: /mod_base= OTHER
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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
30 ATAAGCTTAA NNTNGCNNTN GCNGAC

(2) INFORMATION FOR SEQ ID NO:4:
   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 25 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear
   (ii) MOLECULE TYPE: cDNA
   (ix) FEATURE:
      (A) NAME/KEY: modified_base
      (B) LOCATION: 9
      (D) OTHER INFORMATION: /mod_base= OTHER
          /note= "g or c"
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(A) NAME/KEY: modified_base
(B) LOCATION: 11
(D) OTHER INFORMATION: /mod_base= OTHER
  /note= "g or c"

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(A) NAME/KEY: modified_base
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(A) NAME/KEY: modified_base
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(A) NAME/KEY: modified_base
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(D) OTHER INFORMATION: /mod_base= OTHER
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(A) NAME/KEY: modified_base
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(D) OTHER INFORMATION: /mod_base= OTHER
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25 (ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 21
(D) OTHER INFORMATION: /mod_base= OTHER
  /note= "g or c"

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(A) NAME/KEY: modified_base
(B) LOCATION: 22
(D) OTHER INFORMATION: /mod_base= OTHER
  /note= "a or g"

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
TCGAATTCA NNNTNTANGG NNCAA

(2) INFORMATION FOR SEQ ID NO:5:
  (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 999 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: double
    (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
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TTAGCCTTTG CTGCATTACT ACAAGTACCT TCCTTGGCCAC TGAGATCTTT CTACTACTTTG
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AACATGTATG CAAGACTCTCA CTTTCTGAGT TGCACTGATG TGGACGATG TTGTTTCTC
ATGTAACCCCT TTGCTTCTCA TGACTGCRAA CAGAAATATG ACCTGTACAT CAGCATTTGT
40
GGCTGCGTGA TCATCGCTCA TCCTTGGTTA CTCTTCTTTC ATGCTTTCAC TCCTACTCTG
AGCTATGATC
15
ACCCCTGGCA ATAGGACCAA ATGCTTTGGT GATCTTTCCA CCGAATATG CAACTTGGA
CAGTCCGTGTT TTAATGATGAC CATGGCGAG TGGATGGGT TTGAATCTCC GCTTGTGATT
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ATTGCTTTTG CACCTCTATCA TTTCGATTTT CCTATGATT TCTCGGTGAA GTCCAACTGAA
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ATTAAAGCT GCTTACGGCA AAGGGTGGATT CTAATATTTT CACTCTGTGC ATGTTGTCTTT
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AGTAACATTAG CAGCTCTCAG ATGACACCTG GAATTATGC
15
(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
CGGATTGTGA ATTATGTGTT GAACCATCAT TAGGCAAGAGA TAGCTTTCTCT AGAGAGAATC

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 554 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
TAAAACAAAG AACAAACTCG AATGGACTCT GAAATGCAAG TACCATGAGA CRATCTGCGA
ATACCCAGCG CACAGGGAAAG AACTTGCAGAA ACAACACAGC TTTTACCTTC TGCTCTATCT
TACTGTATG GGGAAATCTCA TTCTCTCAAG CAGGACCTAT TTGGACGATT AGCATCCGAC
ATTATGATAG TGACATGATG CAGTAGTAA TTTTCTCTCA AGTGAGAAA TTTTTTAAATA
TCCTATTTCT GTGACATCGCT ATTAAACATG TGGATCGACACT TTGTTTACCTG
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TGAACCTCAG GCAAAAAGG ATATTAGCTT TGGACTCACC ATACAACTTT AGCTTTTGGG
CAAGAATCG TGGACTTTTA TTGTCAGAGG AAAAAAATG GCTTTTTAT ATTAGTAATC
420
ACAGTTTACA TGCCATTTTC ATATGTTTGG TTATATTTTA GTGGGATAGA TGATATATTA
CCCTGAGTAA AGAAAAATGA AACATAAGAT CATTTTATTC TCTCAAGTGT GATTACTCTT
CAGACGTTCGA ACCG

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(x) SEQUENCE DESCRIPTION: SEQ ID NO:8:
CCAGCAGCCA TGGACTACAA GGACGACGAC GACAAA

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(x) SEQUENCE DESCRIPTION: SEQ ID NO:9:
Trp Phe Leu Met Tyr Pro Phe Arg Phe His Asp Cys Lys Gln Lys Tyr
Asp Leu Tyr Ile

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(x) SEQUENCE DESCRIPTION: SEQ ID NO:10:
Pro Leu Leu Arg Thr Ser Asp Asp Thr Ser Gly Asn Arg Thr Lys Cys
Phe Val Asp Leu Pro Thr Arg Asn Val
CLAIMS

1. A nucleotide sequence consisting essentially of SEQUENCE I.D. NO: 1.


3. Recombinant DNA comprising the nucleotide sequence according to claim 1.

4. Recombinant DNA comprising a nucleotide sequence according to claim 2.

5. Recombinant DNA according to claim 3 wherein the nucleotide sequence is functionally linked to a promoter.

6. Recombinant DNA according to claim 4 wherein the nucleotide sequence is functionally linked to a promoter.

7. A vector comprising recombinant DNA according to claim 3.

8. A vector comprising recombinant DNA according to claim 4.

9. A vector comprising recombinant DNA according to claim 5.

10. A vector comprising recombinant DNA according to claim 6.

11. A transformed or transfected host cell comprising a nucleotide sequence according to claim 1.

12. A transformed or transfected host cell comprising a nucleotide sequence according to claim 2.

13. A nucleotide sequence consisting essentially of SEQUENCE I.D. NO: 5.


15. Recombinant DNA comprising a nucleotide sequence according to claim 13.

16. Recombinant DNA comprising a nucleotide sequence according to claim 14.

17. Recombinant DNA according to claim 15 wherein the nucleotide sequence is functionally linked to a promoter.

18. Recombinant DNA according to claim 16 wherein the nucleotide sequence is functionally linked to a promoter.

19. A vector comprising recombinant DNA according to claim 15.

20. A vector comprising recombinant DNA according to claim 16.
21. A vector comprising recombinant DNA according to claim 17.

22. A vector comprising recombinant DNA according to claim 18.

23. A transformed or transfected host cell comprising a nucleotide sequence according to claim 13.

24. A transformed or transfected host cell comprising a nucleotide sequence according to claim 14.


27. A substantially pure polypeptide of SEQUENCE I.D. NO: 2.


29. An antibody to a polypeptide according to claim 27.

30. An antibody to a polypeptide according to claim 28.

31. An antibody according to claim 29 wherein the antibody is a monoclonal antibody.

32. An antibody according to claim 30 wherein the antibody is a monoclonal antibody.

33. A substantially pure ligand which binds to a polypeptide according to claim 27.

34. A substantially pure ligand which binds to a polypeptide according to claim 28.

35. A method to assay an agent for the ability thereof to act as a ligand or an agonist of a cell receptor comprising a polypeptide according to claim 27, a functional equivalent thereof, a functional fragment of a polypeptide according to claim 27, or a functional equivalent of a functional fragment of a polypeptide according to claim 27, which method comprises:

(a) incubating cells transfected with and capable of expressing SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5, or a functional equivalent thereof, a functional fragment thereof or a functional equivalent of a functional fragment thereof, in the presence of a solubilized chemical entity, a natural product, a purified factor or a crude cellular extract;

(b) incubating control cells of the same type as in step (a), but which are not so transfected, in the presence of the same solubilized chemical entity, a natural product, a purified factor or a crude cellular extract;
entity, natural product, purified factor or crude cellular extract as employed in step (a);

(c) assaying the effect of the solubilized chemical entity, natural product, purified factor or crude cellular extract on the cells of steps (a) and (b); and

(d) determining whether the solubilized chemical entity, natural product, purified factor or crude cellular extract increase or stimulate activity of the cell receptor comprising a polypeptide according to claim 27 for the cells of step (a) when compared to the cells of step (b).

36. The method according to claim 35 wherein, in step (c), the effect is assayed by measuring calcium flux.

37. The method according to claim 35 wherein, in step (a), the transfected cells are co-transfected with a vector encoding luciferase under control of a G-protein sensitive promoter; in step (b), the cells are transfected with the vector containing luciferase under control of G-protein sensitive promoter which is used to co-transfect the cells of step (a); and, in step (c), the effect is assayed by measuring luciferase activity.

38. A method to assay an agent for the ability thereof to act as a selective antagonist of a cell receptor comprising a polypeptide according to claim 27, a functional equivalent thereof, a functional fragment of a polypeptide according to claim 27, or a functional equivalent of a functional fragment of a polypeptide according to claim 27, which method comprises:

(a) separately incubating cells transfected with and capable of expressing

SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5, or a functional equivalent thereof, a functional fragment thereof or a functional equivalent of a functional fragment thereof, and cells containing a known G-protein coupled receptor in the presence of a chemical entity;

(b) adding to the incubations of step (a) a natural ligand or agonist of

SEQUENCE I.D. NO: 2, or a functional equivalent thereof, a functional fragment thereof or a functional equivalent of a functional fragment thereof, to the stably transfected cells and a natural ligand or agonist to the known G-protein coupled receptor cells;

(c) assaying the effect of the chemical entity on the cells; and
(d) determining whether the chemical entity selectively inhibits or blocks the natural ligand or agonist of SEQUENCE I.D. NO: 2, or a functional equivalent thereof, a functional fragment thereof or a functional equivalent of a functional fragment thereof.

39. The method according to claim 38 wherein, in step (c), the effect is assayed by measuring calcium flux.

40. The method according to claim 38 wherein, in step (a), the cells are co-transfected and the cells containing a known G-protein coupled receptor are transfected with a vector encoding luciferase under control of a G-protein sensitive promoter; and, in step (c), the effect is assayed by measuring luciferase activity.

41. A method to assay an agent for the ability thereof to act as an antagonist of a cell receptor comprising a polypeptide according to claim 27, a functional equivalent thereof, a functional fragment of a polypeptide according to claim 27, or a functional equivalent of a functional fragment of a polypeptide according to claim 27, which method comprises:

(a) separately incubating transfected cells capable of expressing SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5, or a functional equivalent thereof, a functional fragment thereof or a functional equivalent of a functional fragment thereof, and control cells of the same type but transfected with and capable of expressing a control receptor other than a receptor comprising a polypeptide according to claim 27 with varying concentrations of a chemical entity;

(b) adding to the respective cells of step (a) a labeled ligand of the respective receptors thereof; and

(c) determining whether the chemical entity blocks or inhibits the binding of the labeled ligand to SEQUENCE I.D. NO: 1 or SEQUENCE I.D. NO: 5, or a functional equivalent thereof, a functional fragment thereof or a functional equivalent of a functional fragment thereof, transfected cells but not to the control cells.

42. A transgenic non-human animal comprising a nucleotide sequence according to claim 1.

43. A transgenic non-human animal comprising a nucleotide sequence according to claim 13.
44. An antisense sequence, a functional equivalent thereof, a functional fragment of an antisense sequence, or a functional equivalent of a functional fragment of an antisense sequence, wherein the antisense sequence is an antisense sequence to an expression sequence of a nucleotide sequence according to claim 1.

45. An antisense sequence, a functional equivalent thereof, a functional fragment of an antisense sequence, or a functional equivalent of a functional fragment of an antisense sequence, wherein the antisense sequence is an antisense sequence to an expression sequence of a nucleotide sequence according to claim 2.

46. An antisense sequence, a functional equivalent thereof, a functional fragment of an antisense sequence, or a functional equivalent of a functional fragment of an antisense sequence, wherein the antisense sequence is an antisense sequence to an expression sequence of a nucleotide sequence according to claim 13.

47. An antisense sequence, a functional equivalent thereof, a functional fragment of an antisense sequence, or a functional equivalent of a functional fragment of an antisense sequence, wherein the antisense sequence is an antisense sequence to an expression sequence of a nucleotide sequence according to claim 14.

48. A modified oligonucleotide which binds to an antisense sequence, a functional equivalent thereof, a functional fragment of an antisense sequence or a functional equivalent of a functional fragment of an antisense sequence according to claim 44.

49. A modified oligonucleotide which binds to an antisense sequence, a functional equivalent thereof, a functional fragment of an antisense sequence or a functional equivalent of a functional fragment of an antisense sequence according to claim 45.

50. A modified oligonucleotide which binds to an antisense sequence, a functional equivalent thereof, a functional fragment of an antisense sequence or a functional equivalent of a functional fragment of an antisense sequence according to claim 46.

51. A modified oligonucleotide which binds to an antisense sequence, a functional equivalent thereof, a functional fragment of an antisense sequence or a functional equivalent of a functional fragment of an antisense sequence according to claim 47.

52. A composition comprising a modified oligonucleotide according to claim 48 and the antisense sequence, functional equivalent, functional fragment of an
antisense sequence or the functional equivalent of a functional fragment of an antisense sequence to which it binds.

53. A composition comprising a modified oligonucleotide according to claim 49 and the antisense sequence, functional equivalent, functional fragment of an antisense sequence or the functional equivalent of a functional fragment of an antisense sequence to which it binds.

54. A composition comprising a modified oligonucleotide according to claim 50 and the antisense sequence, functional equivalent, functional fragment of an antisense sequence or the functional equivalent of a functional fragment of an antisense sequence to which it binds.

55. A composition comprising a modified oligonucleotide according to claim 51 and the antisense sequence, functional equivalent, functional fragment of an antisense sequence or the functional equivalent of a functional fragment of an antisense sequence to which it binds.
FIG. 1

pCRII/Da6
5513bp
### INTERNATIONAL SEARCH REPORT

**Interr: al Application No**

**PCT/US 94/00598**

#### A. CLASSIFICATION OF SUBJECT MATTER

<table>
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<th>IPC</th>
<th>C12N5/12</th>
<th>C07K13/00</th>
<th>C12N5/10</th>
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<th>C12P21/08</th>
<th>C12Q1/68</th>
<th>G01N33/68</th>
<th>A01K67/027</th>
<th>C07H21/00</th>
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According to International Patent Classification (IPC) or to both national classification and IPC

#### B. FIELDS SEARCHED

**Minimum documentation searched (classification system followed by classification symbols)**

| IPC | C12N | C07K | C12Q | G01N | A01K |

**Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched**

**Electronic data base consulted during the international search (name of data base and, where practical, search terms used)**

#### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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<tbody>
<tr>
<td>A</td>
<td>CURRENT OPINION IN STRUCTURAL BIOLOGY vol. 1, no. 3, June 1991 pages 394 - 401 KERLAVAGE, A.R.; 'G-protein coupled receptor family' See page 398, &quot;Use of polymerase chain reaction to isolate new receptor genes.&quot;</td>
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</tr>
<tr>
<td>A</td>
<td>TRENDS IN PHARMACOLOGICAL SCIENCES vol. 11, December 1990, CAMBRIDGE GB pages 492 - 499 FINDLAY, J. ET AL.; 'Three-dimensional modelling of G protein-linked receptors.'</td>
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</table>

**X** Further documents are listed in the continuation of box C. **□** Patent family members are listed in annex.

**"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)  
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**"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 when the document is taken alone  
**"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  
**"Y"** document member of the same patent family

**Date of the actual completion of the international search**

27 May 1994

**Date of mailing of the international search report**

14.06.94

**Name and mailing address of the ISA**

European Patent Office, P.B. 5818 Patentiaan 2 NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+31-70) 340-3016

**Authorized officer**

Nauche, S
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<tr>
<td>A</td>
<td>SCIENCE vol. 240, 10 June 1988, LANCASTER, PA pages 1468 - 1474 JAENISCH, R.; 'Transgenic animals' see the whole document</td>
<td>42,43</td>
</tr>
<tr>
<td>P,A</td>
<td>SCIENCE vol. 261, 20 August 1993, LANCASTER, PA pages 1004 - 1012 STEIN, C.A. ET AL.; 'Antisense oligonucleotides as therapeutic agents - Is the bullet really magical?' see the whole document</td>
<td>44-55</td>
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