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(54) Title: REGULATION OF HUMAN CYSLT2-LIKE GPCR PROTEIN

(57) Abstract: Reagents which regulate human CysLT2-like GPCR protein and reagents which bind to human CysLT2-like GPCR gene products can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to peripheral and central nervous system disease, asthma and cardiovascular disease.

REGULATION OF HUMAN CYSLT2-LIKE GPCR PROTEIN

TECHNICAL FIELD OF THE INVENTION

The invention relates to the area of G-protein coupled receptors. More particularly, it relates to the area of CysLT2-like GPCR proteins and their regulation.

BACKGROUND OF THE INVENTION

G-Protein Coupled Receptors

Many medically significant biological processes are mediated by signal transduction pathways that involve G-proteins (Lefkowitz, *Nature 351*, 353-354, 1991). The family of G-protein coupled receptors (GPCR) includes receptors for hormones, neurotransmitters, growth factors, and viruses. Specific examples of GPCRs include receptors for such diverse agents as dopamine, calcitonin, adrenergic hormones, endothelin, cAMP, adenosine, acetylcholine, serotonin, histamine, thrombin, kinin, follicle stimulating hormone, opsins, endothelial differentiation gene-1, rhodopsins, odorants, cytomegalovirus, G-proteins themselves, effector proteins such as phospholipase C, adenyl cyclase, and phosphodiesterase, and actuator proteins such as protein kinase A and protein kinase C.

GPCRs possess seven conserved membrane-spanning domains connecting at least eight divergent hydrophilic loops. GPCRs (also known as 7TM receptors) have been characterized as including these seven conserved hydrophobic stretches of about 20 to 30 amino acids, connecting at least eight divergent hydrophilic loops. Most GPCRs have single conserved cysteine residues in each of the first two extracellular loops, which form disulfide bonds that are believed to stabilize functional protein structure. The seven transmembrane regions are designated as TM1, TM2, TM3, TM4, TM5, TM6, and TM7. TM3 has been implicated in signal transduction.

Phosphorylation and lipidation (palmitylation or farnesylation) of cysteine residues can influence signal transduction of some GPCRs. Most GPCRs contain potential phosphorylation sites within the third cytoplasmic loop and/or the carboxy terminus. For several GPCRs, such as the β-adrenergic receptor, phosphorylation by protein kinase A and/or specific receptor kinases mediates receptor desensitization.

For some receptors, the ligand binding sites of GPCRs are believed to comprise hydrophilic sockets formed by several GPCR transmembrane domains. The hydrophilic sockets are surrounded by hydrophobic residues of the GPCRs. The hydrophilic side of each GPCR transmembrane helix is postulated to face inward and form a polar ligand binding site. TM3 has been implicated in several GPCRs as having a ligand binding site, such as the TM3 aspartate residue. TM5 serines, a TM6 asparagine, and TM6 or TM7 phenylalanines or tyrosines also are implicated in ligand binding.

GPCRs are coupled inside the cell by heterotrimeric G-proteins to various intracellular enzymes, ion channels, and transporters (*see* Johnson *et al.*, *Endoc. Rev. 10*, 317-331, 1989). Different G-protein alpha-subunits preferentially stimulate particular effectors to modulate various biological functions in a cell. Phosphorylation of cytoplasmic residues of GPCRs is an important mechanism for the regulation of some GPCRs. For example, in one form of signal transduction, the effect of hormone binding is the activation inside the cell of the enzyme, adenylate cyclase. Enzyme activation by hormones is dependent on the presence of the nucleotide GTP. GTP also influences hormone binding. A G-protein connects the hormone receptor to adenylate cyclase. G-protein exchanges GTP for bound GDP when activated by a hormone receptor. The GTP-carrying form then binds to activated adenylate cyclase. Hydrolysis of GTP to GDP, catalyzed by the G-protein itself, returns the G-protein to its basal, inactive form. Thus, the G-protein serves a dual role, as an intermediate that relays the signal from receptor to effector, and as a clock that controls the duration of the signal.

Over the past 15 years, nearly 350 therapeutic agents targeting GPCRs receptors have been successfully introduced onto the market. This indicates that these receptors have an established, proven history as therapeutic targets. Clearly, there is an ongoing need for identification and characterization of further GPCRs which can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, infections such as bacterial, fungal, protozoan, and viral infections, particularly those caused by HIV viruses, cancers, anorexia, bulimia, asthma, , acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, angina pectoris, myocardial infarction, ulcers, asthma, allergies, multiple sclerosis, benign prostatic hypertrophy, and GPCRs are of critical importance to both central and peripheral nervous system and novel GPCRs are therefore promising new targets for the treatment of nervous system disease, for example in primary and secondary disorders after brain injury, disorders of mood, anxiety disorders, disorders of thought and volition, disorders of sleep and wakefulness, diseases of the motor unit like neurogenic and myopathic disorders, neurodegenerative disorders like Alzheimer's and Parkinson's disease, disorders leading to peripheral and chronic pain. Since CysLT2 receptors play a role in inflammation CysLT2-like GPCR could be important especially in inflammatory diseases of the nervous system like inflammatory pain, arthritis, multiple sclerosis etc.

Because of the wide-spread distribution of GCPRs with diverse biological effects, there is a need in the art to identify additional members of the GCPR family whose activity can be regulated to provide therapeutic effects.

SUMMARY OF THE INVENTION

It is an object of the invention to provide reagents and methods of regulating a CysLT2-like GPCR protein. This and other objects of the invention are provided by one or more of the embodiments described below.

One embodiment of the invention is a CysLT2-like GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 39% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

Yet another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a CysLT2-like GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 39% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

Binding between the test compound and the CysLT2-like GPCR polypeptide is detected. A test compound which binds to the CysLT2-like GPCR polypeptide is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a polynucleotide encoding a CysLT2-like GPCR polypeptide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 39% identical to the nucleotide sequence shown in SEQ ID NO: 1;

the nucleotide sequence shown in SEQ ID NO: 1;

nucleotide sequences which are at least about 39% identical to the nucleotide sequence shown in SEQ ID NO: 3; and

the nucleotide sequence shown in SEQ ID NO: 3.

Binding of the test compound to the polynucleotide is detected. A test compound which binds to the polynucleotide is identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the amount of the CysLT2-like GPCR through interacting with the CysLT2-like GPCR mRNA.

Another embodiment of the invention is a method of screening for agents which regulate extracellular matrix degradation. A test compound is contacted with a CysLT2-like GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 39% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

A CysLT2-like GPCR activity of the polypeptide is detected. A test compound which increases CysLT2-like GPCR activity of the polypeptide relative to CysLT2-like GPCR activity in the absence of the test compound is thereby identified as a potential agent for increasing extracellular matrix degradation. A test compound which decreases CysLT2-like GPCR activity of the polypeptide relative to CysLT2-like GPCR activity in the absence of the test compound is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Even another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a

CysLT2-like GPCR product of a polynucleotide which comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 39% identical to the nucleotide sequence shown in SEQ ID NO: 1;

the nucleotide sequence shown in SEQ ID NO: 1;

nucleotide sequences which are at least about 39% identical to the nucleotide sequence shown in SEQ ID NO: 3; and

the nucleotide sequence shown in SEQ ID NO: 3.

Binding of the test compound to the CysLT2-like GPCR product is detected. A test compound which binds to the CysLT2-like GPCR product is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Still another embodiment of the invention is a method of reducing extracellular matrix degradation. A cell is contacted with a reagent which specifically binds to a polynucleotide encoding a CysLT2-like GPCR polypeptide or the product encoded by the polynucleotide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 39% identical to the nucleotide sequence shown in SEQ ID NO: 1;

the nucleotide sequence shown in SEQ ID NO: 1;

nucleotide sequences which are at least about 39% identical to the nucleotide sequence shown in SEQ ID NO: 3; and

the nucleotide sequence shown in SEQ ID NO: 3.

CysLT2-like GPCR activity in the cell is thereby decreased.

The invention thus provides a CysLT2-like GPCR which can be used to identify test compounds for human GPCR **MODULATORS SUCH AS** agonists and antagonists, partial agonist, inverse agonist, co-activators.CysLT2-like GPCR protein and fragments thereof also are useful in raising specific antibodies which can block the receptor and effectively prevent ligand binding.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. BLASTP alignment of 38_TR1 (SEQ ID NO:2) against swiss|Q13304|GPRH_HUMAN (SEQ ID NO:3).

FIG. 2. BLOCKS search results.

FIG. 3. BLASTP alignment of SEQ ID NO:2 against trembl|AF119711|AF119711_1.

FIG. 4. BLASTPalignment of SEQ ID NO:2 against trembl|Y12546|HSP2YLG_1.

FIG. 5. Relative expression of human CysLT2-like GPCR in respiratory cells and tissues.

FIG. 6. Relative expression of human CysLT2-like GPCR in various human tissues and the neutrophil-like cell line HL60.

FIG. 7. Amino acid sequence and transmembrane domains of human CysLT2-like GPCR.

FIG. 8. DNA-sequence encoding CysLT2-like GPCR polypeptide.

FIG.9. Amino acid sequence deduced from the DNA-sequence of FIG. 8.

FIG 10. DNA-sequence encoding CysLT2-like GPCR polypeptide.

FIG. 11. Quantitative expression of human CysLT2-like GPCR (A) in comparison with CysLT1 GPCR (B).

FIG. 12, 13, 14 Quantitative expression of human CysLT2-like GPCR in specific tissues and organs.

FIG. 15, 16, 17 Effects of CysLT2-like GPCR antagonists on calcium mobilization of cells.

FIG. 18, 19, 20 Binding and inhibited binding of specific molecules to CysLT2-like GPCR.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to an isolated polynucleotide encoding a CysLT2-like GPCR polypeptide and being selected from the group consisting of:

a) a polynucleotide encoding a CysLT2-like GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:
amino acid sequences which are at least about 39% identical to
the amino acid sequence shown in SEQ ID NO: 2; and
the amino acid sequence shown in SEQ ID NO: 2.

b) a polynucleotide comprising the sequence of SEQ ID NOS: 1 or 3;

c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);

d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and

e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d).

Furthermore, it has been discovered by the present applicant that a CysLT2-like GPCR protein, particularly a human CysLT2-like GPCR protein, can be used in therapeutic methods to treat disorders such as bacterial, fungal, protozoan, and viral infections, particularly those caused by HIV viruses, cancers, anorexia, bulimia, COPD, cardiovascular disease such as acute heart failure, angina pectoris,

myocardial infarction, hypotension and hypertension, urinary retention, osteoporosis, ulcers, asthma, allergies, benign prostatic hypertrophy, and GPCRs are of critical importance to both central and peripheral nervous system and novel GPCRs are therefore promising new targets for the treatment of nervous system disease, for example in primary and secondary disorders after brain injury, disorders of mood, anxiety disorders, disorders of thought and volition, disorders of sleep and wakefulness, diseases of the motor unit like neurogenic and myopathic disorders, neurodegenerative disorders like Alzheimer's and Parkinson's disease, disorders leading to peripheral and chronic pain. Since CysLT2 receptors play a role in inflammation CysLT2-like GPCR could be important especially in inflammatory diseases of the nervous system like inflammatory pain, arthritis, multiple sclerosis etc.

Human CysLT2-like GPCR also can be used to screen for CysLT2-like GPCR agonists and antagonists. partial agonist, inverse agonist, co-activators

Human CysLT2-like GPCR is 35% identical over 317 amino acids to swiss|Q13304|GPRH_HUMAN (FIG. 1), 38% identical over 298 amino acids to trembl|AF119711|AF119711_1 (FIG. 3), and 34% identical over 322 amino acids to against trembl|Y12546|HSP2YLG_1. Human CysLT2-like GPCR is homologous to cysteinyl leukotriene (cycLT1 LTD4) receptor and to P2Y receptors.

CysLT2-like GPCR Polypeptides

CysLT2-like GPCR polypeptides according to the invention comprise an amino acid sequence shown in SEQ ID NO:2 a portion of that sequence, or a biologically active variant thereof, as defined below. A CysLT2-like GPCR polypeptide of the invention therefore can be a portion of a CysLT2-like GPCR protein, a full-length CysLT2-like GPCR protein, or a fusion protein comprising all or a portion of a CysLT2-like GPCR protein. The amino acid sequence shown in SEQ ID NO:2 contains a transmembrane helix from amino acids 93-110 and from amino acids 115-

139. The complement of a nucleotide coding sequence for SEQ ID NO: 2 is shown in SEQ ID NO: 1.

Biologically Active Variants

CysLT2-like GPCR polypeptide variants which are biologically active, *i.e.*, retain the ability to bind a ligand to produce a biological effect, such as cyclic AMP formation, mobilization of intracellular calcium, or phosphoinositide metabolism, also are CysLT2-like GPCR polypeptides. Preferably, naturally or non-naturally occurring CysLT2-like GPCR polypeptide variants have amino acid sequences which are at least about 39, 40, 45, 50, preferably about 75, 90, 96, or 98% identical to an amino acid sequence shown in SEQ ID NO:2 or a fragment thereof. Percent identity between a putative CysLT2-like GPCR polypeptide variant and an amino acid sequence of SEQ ID NO:2 is determined using the Blast2 alignment program.

Variations in percent identity can be due, for example, to amino acid substitutions, insertions, or deletions. Amino acid substitutions are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity of a CysLT2-like GPCR polypeptide can be found using computer programs well known in the art, such as DNASTAR software. Whether an amino acid change results in a biologically active CysLT2-like GPCR polypeptide can readily be determined by assaying for binding to a ligand or by conducting a functional assay, as described for example, in the specific Examples, below.

Fusion Proteins

Fusion proteins can comprise at least 5, 6, 8, 10, 25, or 50 or more contiguous amino acids of an amino acid sequence shown in SEQ ID NO:2. Fusion proteins are useful for generating antibodies against CysLT2-like GPCR polypeptide amino acid sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins which interact with portions of a CysLT2-like GPCR polypeptide. Protein affinity chromatography or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art and also can be used as drug screens.

A CysLT2-like GPCR polypeptide fusion protein comprises two polypeptide segments fused together by means of a peptide bond. The first polypeptide segment comprises at least 5, 6, 8, 10, 25, or 50 or more contiguous amino acids of SEQ ID NO:2. Contiguous amino acids for use in a fusion protein can be selected from the amino acid sequence shown in SEQ ID NO:2 or from a biologically active variant of those sequences, such as those described above. The first polypeptide segment also can comprise full-length CysLT2-like GPCR protein.

The second polypeptide segment can be a full-length protein or a protein fragment. Proteins commonly used in fusion protein construction include β-galactosidase, β-glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions.

A fusion protein also can be engineered to contain a cleavage site located between the CysLT2-like GPCR polypeptide-encoding sequence and the heterologous protein sequence, so that the CysLT2-like GPCR polypeptide can be cleaved and purified away from the heterologous moiety.

A fusion protein can be synthesized chemically, as is known in the art. Preferably, a fusion protein is produced by covalently linking two polypeptide segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises coding sequences selected from the complement of SEQ ID NO:1 in proper reading frame with nucleotides encoding the second polypeptide segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies such as Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), CLONTECH (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Identification of Species Homologs

Species homologs of human CysLT2-like GPCR polypeptide can be obtained using CysLT2-like GPCR polypeptide polynucleotides (described below) to make suitable probes or primers for screening cDNA expression libraries from other species, such as mice, monkeys, or yeast, identifying cDNAs which encode homologs of CysLT2-like GPCR polypeptide, and expressing the cDNAs as is known in the art.

CysLT2-like GPCR Polynucleotides

A CysLT2-like GPCR polynucleotide can be single- or double-stranded and comprises a coding sequence or the complement of a coding sequence for a CysLT2-like GPCR polypeptide. A coding sequence for CysLT2-like GPCR is shown in

SEQ ID NO:1; this coding sequence is located in the longer sequence shown in SEQ ID NO:3.

Degenerate nucleotide sequences encoding human CysLT2-like GPCR polypeptides, as well as homologous nucleotide sequences which are at least about 50, preferably about 75, 90, 96, or 98% identical to the nucleotide sequence shown in SEQ ID NO:1 or 3 or their complements also are CysLT2-like GPCR polynucleotides. Percent sequence identity between the sequences of two polynucleotides is determined using computer programs such as ALIGN which employ the FASTA algorithm, using an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2. Complementary DNA (cDNA) molecules, species homologs, and variants of CysLT2-like GPCR polynucleotides which encode biologically active CysLT2-like GPCR polypeptides also are CysLT2-like GPCR polynucleotides, as are polynucleotides comprising at least 6, 7, 8, 9, 10, 12, 15, 18, 20, or 25 contiguous nucleotides of SEQ ID NO:1 or its complement. Such polynucleotides can be used, for example, as hybridization probes or as antisense oligonucleotides.

Identification of Variants and Homologs

Variants and homologs of the CysLT2-like GPCR polynucleotides described above also are CysLT2-like GPCR polynucleotides. Typically, homologous CysLT2-like GPCR polynucleotide sequences can be identified by hybridization of candidate polynucleotides to known CysLT2-like GPCR polynucleotides under stringent conditions, as is known in the art. For example, using the following wash conditions--2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50 μC once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each--homologous sequences can be identified which contain at most about 25-30% basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

Species homologs of the CysLT2-like GPCR polynucleotides disclosed herein also can be identified by making suitable probes or primers and screening cDNA expression libraries from other species, such as mice, monkeys, or yeast. Human variants of CysLT2-like GPCR polynucleotides can be identified, for example, by screening human cDNA expression libraries. It is well known that the T_m of a double-stranded DNA decreases by 1-1.5 °C with every 1% decrease in homology (Bonner *et al.*, *J. Mol. Biol. 81*, 123 (1973). Variants of human CysLT2-like GPCR polynucleotides or CysLT2-like GPCR polynucleotides of other species can therefore be identified by hybridizing a putative homologous CysLT2-like GPCR polynucleotide with a polynucleotide having a nucleotide sequence of SEQ ID NO:1 or the complement thereof to form a test hybrid. The melting temperature of the test hybrid is compared with the melting temperature of a hybrid comprising transformylase polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is calculated.

Nucleotide sequences which hybridize to transformylase polynucleotides or their complements following stringent hybridization and/or wash conditions also are CysLT2-like GPCR polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed., 1989, at pages 9.50-9.51.

Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20 °C below the calculated T_m of the hybrid under study. The T_m of a hybrid between a CysLT2-like GPCR polynucleotide having a nucleotide sequence shown in SEQ ID NO:1 or the complement thereof and a polynucleotide sequence which is at least about 50, preferably about 75, 90, 96, or 98% identical to one of those nucleotide sequences can be calculated, for example, using the equation of Bolton and McCarthy, *Proc. Natl. Acad. Sci. U.S.A. 48*, 1390 (1962):

$T_m = 81.5\ ^{\circ}C - 16.6(\log_{10}[Na^+]) + 0.41(\%G + C) - 0.63(\%formamide) - 600/l$, where l = the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4X SSC at 65 °C, or 50% formamide, 4X SSC at 42 °C, or 0.5X SSC, 0.1% SDS at 65 °C. Highly stringent wash conditions include, for example, 0.2X SSC at 65 °C.

Preparation of CysLT2-like GPCR Polynucleotides

A naturally occurring CysLT2-like GPCR polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, or synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a polynucleotide can be used to obtain isolated CysLT2-like GPCR polynucleotides. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments which comprises CysLT2-like GPCR nucleotide sequences. Isolated polynucleotides are in preparations which are free or at least 70, 80, or 90% free of other molecules.

CysLT2-like GPCR cDNA molecules can be made with standard molecular biology techniques, using CysLT2-like GPCR mRNA as a template. CysLT2-like GPCR cDNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in manuals such as Sambrook *et al.* (1989). An amplification technique, such as PCR, can be used to obtain additional copies of polynucleotides of the invention, using either human genomic DNA or cDNA as a template.

Alternatively, synthetic chemistry techniques can be used to synthesizes CysLT2-like GPCR polynucleotides. The degeneracy of the genetic code allows alternate

nucleotide sequences to be synthesized which will encode a CysLT2-like GPCR polypeptide having, for example, an amino acid sequence shown in SEQ ID NO:2 or a biologically active variant thereof.

Extending CysLT2-like GPCR Polynucleotides

Various PCR-based methods can be used to extend the nucleic acid sequences disclosed herein to detect upstream sequences such as promoters and regulatory elements. For example, restriction-site PCR uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, *PCR Methods Applic. 2*, 318-322, 1993). Genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR also can be used to amplify or extend sequences using divergent primers based on a known region (Triglia *et al.*, *Nucleic Acids Res. 16*, 8186, 1988). Primers can be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, Minn.), to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72 °C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which can be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom *et al.*, *PCR Methods Applic. 1*, 111-119, 1991). In this method, multiple restriction enzyme digestions and ligations also can

be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

Another method which can be used to retrieve unknown sequences is that of Parker *et al.*, *Nucleic Acids Res. 19*, 3055-3060, 1991). Additionally, PCR, nested primers, and PROMOTERFINDER libraries (CLONTECH, Palo Alto, Calif.) can be used to walk genomic DNA (CLONTECH, Palo Alto, Calif.). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Randomly-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries can be useful for extension of sequence into 5' non-transcribed regulatory regions.

Commercially available capillary electrophoresis systems can be used to analyze the size or confirm the nucleotide sequence of PCR or sequencing products. For example, capillary sequencing can employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) that are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity can be converted to electrical signal using appropriate software (*e.g.* GENOTYPER and Sequence NAVIGATOR, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display can be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA that might be present in limited amounts in a particular sample.

Obtaining CysLT2-like GPCR Polypeptides

CysLT2-like GPCR polypeptides can be obtained, for example, by purification from human cells, by expression of CysLT2-like GPCR polynucleotides, or by direct chemical synthesis.

Protein Purification

CysLT2-like GPCR polypeptides can be purified from any human cell which expresses the receptor, including host cells which have been transfected with CysLT2-like GPCR polynucleotides. A purified CysLT2-like GPCR polypeptide is separated from other compounds which normally associate with the CysLT2-like GPCR polypeptide in the cell, such as certain proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis.

CysLT2-like GPCR polypeptide can be conveniently isolated as a complex with its associated G protein, as described in the specific examples, below. A preparation of purified CysLT2-like GPCR polypeptides is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis.

Expression of CysLT2-like GPCR Polynucleotides

To express a CysLT2-like GPCR polypeptide, a CysLT2-like GPCR polynucleotide can be inserted into an expression vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding CysLT2-like GPCR polypeptides and appropriate transcriptional and translational control elements. These methods include *in vitro*

recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook *et al.* (1989) and in Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y., 1989.

A variety of expression vector/host systems can be utilized to contain and express sequences encoding a CysLT2-like GPCR polypeptide. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors, insect cell systems infected with virus expression vectors (*e.g.*, baculovirus), plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids), or animal cell systems.

The control elements or regulatory sequences are those non-translated regions of the vector -- enhancers, promoters, 5' and 3' untranslated regions -- which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (*e.g.*, heat shock, RUBISCO, and storage protein genes) or from plant viruses (*e.g.*, viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding a CysLT2-like GPCR polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

Bacterial and Yeast Expression Systems

In bacterial systems, a number of expression vectors can be selected depending upon the use intended for the CysLT2-like GPCR polypeptide. For example, when a large quantity of a CysLT2-like GPCR polypeptide is needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified can be used. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene). In a BLUESCRIPT vector, a sequence encoding the CysLT2-like GPCR polypeptide can be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β-galactosidase so that a hybrid protein is produced. pIN vectors (Van Heeke & Schuster, *J. Biol. Chem. 264*, 5503-5509, 1989) or pGEX vectors (Promega, Madison, Wis.) also can be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems can be designed to include heparin, thrombin, or factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH can be used. For reviews, see Ausubel *et al.* (1989) and Grant *et al.*, *Methods Enzymol. 153*, 516-544, 1987.

Plant and Insect Expression Systems

If plant expression vectors are used, the expression of sequences encoding CysLT2-like GPCR polypeptides can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used

alone or in combination with the omega leader sequence from TMV (Takamatsu, *EMBO J. 6*, 307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters can be used (Coruzzi *et al.*, *EMBO J. 3*, 1671-1680, 1984; Broglie *et al.*, *Science 224*, 838-843, 1984; Winter *et al.*, *Results Probl. Cell Differ. 17*, 85-105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (*e.g.*, Hobbs or Murray, in MCGRAW HILL YEARBOOK OF SCIENCE AND TECHNOLOGY, McGraw Hill, New York, N.Y., pp. 191-196, 1992).

An insect system also can be used to express a CysLT2-like GPCR polypeptide. For example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. Sequences encoding CysLT2-like GPCR polypeptides can be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of CysLT2-like GPCR polypeptides will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to infect *S. frugiperda cells* or *Trichoplusia* larvae in which CysLT2-like GPCR polypeptides can be expressed (Engelhard *et al.*, *Proc. Nat. Acad. Sci. 91*, 3224-3227, 1994).

Mammalian Expression Systems

A number of viral-based expression systems can be used to express CysLT2-like GPCR polypeptides in mammalian host cells. For example, if an adenovirus is used as an expression vector, sequences encoding CysLT2-like GPCR polypeptides can be ligated into an adenovirus transcription/translation complex comprising the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus which is capable of expressing a CysLT2-like GPCR polypeptide in infected host cells (Logan & Shenk,

Proc. Natl. Acad. Sci. 81, 3655-3659, 1984). If desired, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) also can be used to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of 6M to 10M are constructed and delivered to cells via conventional delivery methods (*e.g.*, liposomes, polycationic amino polymers, or vesicles).

Specific initiation signals also can be used to achieve more efficient translation of sequences encoding CysLT2-like GPCR polypeptides. Such signals include the ATG initiation codon and adjacent sequences (Kozak sequence). In cases where sequences encoding a CysLT2-like GPCR polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals (including the ATG initiation codon) should be provided. The initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used (see Scharf *et al.*, *Results Probl. Cell Differ. 20*, 125-162, 1994).

Host Cells

A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed CysLT2-like GPCR polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the

polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (*e.g.*, CHO, HeLa, MDCK, HEK293, 1321N1 and WI38), are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

Stable expression is preferred for long-term, high-yield production of recombinant proteins. For example, cell lines can be stably transfected via conventional transfection method, e.g. liposomes, polycationic amino polymers, vesicles, electroporation, calcium-phosphate, etc. using expression vectors which can contain the cloned CysLT2-like GPCR cDNA or genomic DNA, viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched medium before they are switched to a selective medium. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced CysLT2-like GPCR sequences. Resistant clones of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type. See, for example, ANIMAL CELL CULTURE, R.I. Freshney, ed., 1986.

Any number of selection systems can be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell 11*, 223-32, 1977) and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell 22*, 817-23, 1980) genes which can be employed in *tk*$^-$ or *aprt*$^-$ cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler *et al.*, *Proc. Natl. Acad. Sci. 77*, 3567-70, 1980), *npt* confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin *et al.*, *J. Mol. Biol. 150*, 1-14, 1981), and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murray, 1992, *supra*). Additional selectable genes

have been described. For example, *trpB* allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci. 85*, 8047-51, 1988). Visible markers such as anthocyanins, β-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, can be used to identify transformants and to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes *et al.*, *Methods Mol. Biol. 55*, 121-131, 1995).

Detecting Expression of CysLT2-like GPCR Polypeptides

Although the presence of marker gene expression suggests that the CysLT2-like GPCR polynucleotide is also present, its presence and expression may need to be confirmed. For example, if a sequence encoding a CysLT2-like GPCR polypeptide is inserted within a marker gene sequence, transformed cells containing sequences which encode a CysLT2-like GPCR polypeptide can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding a CysLT2-like GPCR polypeptide under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the CysLT2-like GPCR polynucleotide.

Alternatively, host cells which contain a CysLT2-like GPCR polynucleotide and which express a CysLT2-like GPCR polypeptide can be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip-based technologies for the detection and/or quantification of nucleic acid or protein. For example, the presence of a polynucleotide sequence encoding a CysLT2-like GPCR polypeptide can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding a CysLT2-like GPCR polypeptide. Nucleic acid amplification-based assays involve the use of

oligonucleotides selected from sequences encoding a CysLT2-like GPCR polypeptide to detect transformants which contain a CysLT2-like GPCR polynucleotide.

A variety of protocols for detecting and measuring the expression of a CysLT2-like GPCR polypeptide, using either polyclonal or monoclonal antibodies specific for the polypeptide, are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on a CysLT2-like GPCR polypeptide can be used, or a competitive binding assay can be employed. These and other assays are described in Hampton *et al.*, SEROLOGICAL METHODS: A LABORATORY MANUAL, APS Press, St. Paul, Minn., 1990) and Maddox *et al.*, *J. Exp. Med. 158*, 1211-1216, 1983).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding CysLT2-like GPCR polypeptides include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, sequences encoding a CysLT2-like GPCR polypeptide can be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of labeled nucleotides and an appropriate RNA polymerase such as T7, T3, or SP6. These procedures can be conducted using a variety of commercially available kits (Amersham Pharmacia Biotech, Promega, and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, and fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Expression and Purification of CysLT2-like GPCR Polypeptides

Host cells transformed with nucleotide sequences encoding a CysLT2-like GPCR polypeptide can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode CysLT2-like GPCR polypeptides can be designed to contain signal sequences which direct secretion of soluble CysLT2-like GPCR polypeptides through a prokaryotic or eukaryotic cell membrane or which direct the membrane insertion of membrane-bound CysLT2-like GPCR polypeptide.

As discussed above, other constructions can be used to join a sequence encoding a CysLT2-like GPCR polypeptide to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). Inclusion of cleavable linker sequences such as those specific for Factor Xa or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the CysLT2-like GPCR polypeptide also can be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a CysLT2-like GPCR polypeptide and 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification by IMAC (immobilized metal ion affinity chromatography, as described in Porath *et al.*, *Prot. Exp. Purif. 3*, 263-281, 1992), while the enterokinase cleavage site provides a means for purifying the CysLT2-like GPCR polypeptide from the fusion protein. Vectors which contain fusion proteins are disclosed in Kroll *et al.*, *DNA Cell Biol. 12*, 441-453, 1993.

Chemical Synthesis

Sequences encoding a CysLT2-like GPCR polypeptide can be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers *et al.*, *Nucl. Acids Res. Symp. Ser.* 215-223, 1980; Horn *et al. Nucl. Acids Res. Symp. Ser.* 225-232, 1980). Alternatively, a CysLT2-like GPCR polypeptide itself can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc.* *85*, 2149-2154, 1963; Roberge *et al.*, *Science 269*, 202-204, 1995). Protein synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of CysLT2-like GPCR polypeptides can be separately synthesized and combined using chemical methods to produce a full-length molecule.

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES, WH Freeman and Co., New York, N.Y., 1983). The composition of a synthetic CysLT2-like GPCR polypeptide can be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see* Creighton, *supra*). Additionally, any portion of the amino acid sequence of the CysLT2-like GPCR polypeptide can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

Production of Altered CysLT2-like GPCR Polypeptides

As will be understood by those of skill in the art, it may be advantageous to produce CysLT2-like GPCR polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular

prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences disclosed herein can be engineered using methods generally known in the art to alter CysLT2-like GPCR polypeptide-encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the polypeptide or mRNA product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site-directed mutagenesis can be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

Antibodies

Any type of antibody known in the art can be generated to bind specifically to an epitope of a CysLT2-like GPCR polypeptide. "Antibody" as used herein includes intact immunoglobulin molecules, as well as fragments thereof, such as Fab, $F(ab')_2$, and Fv, which are capable of binding an epitope of a CysLT2-like GPCR polypeptide. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, *e.g.*, at least 15, 25, or 50 amino acids.

An antibody which specifically binds to an epitope of a CysLT2-like GPCR polypeptide can be used therapeutically, as well as in immunochemical assays, such as Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various immunoassays can be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays are well

known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody which specifically binds to the immunogen.

Typically, an antibody which specifically binds to a CysLT2-like GPCR polypeptide provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay. Preferably, antibodies which specifically bind to CysLT2-like GPCR polypeptides do not detect other proteins in immunochemical assays and can immunoprecipitate a CysLT2-like GPCR polypeptide from solution.

CysLT2-like GPCR polypeptides can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, a CysLT2-like GPCR polypeptide can be conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin. Depending on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (*e.g.*, aluminum hydroxide), and surface active substances (*e.g.* lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used in humans, BCG (*bacilli Calmette-Guerin*) and *Corynebacterium parvum* are especially useful.

Monoclonal antibodies which specifically bind to a CysLT2-like GPCR polypeptide can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler *et al.*, *Nature 256*, 495-497, 1985; Kozbor *et al.*, *J. Immunol. Methods 81*, 31-42, 1985; Cote *et al.*, *Proc. Natl. Acad. Sci. 80*, 2026-2030, 1983; Cole *et al.*, *Mol. Cell Biol. 62*, 109-120, 1984).

In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al.*, *Proc. Natl. Acad. Sci. 81*, 6851-6855, 1984; Neuberger *et al.*, *Nature 312*, 604-608, 1984; Takeda *et al.*, *Nature 314*, 452-454, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grating of entire complementarity determining regions. Alternatively, humanized antibodies can be produced using recombinant methods, as described in GB2188638B. Antibodies which specifically bind to a CysLT2-like GPCR polypeptide can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies which specifically bind to CysLT2-like GPCR polypeptides. Antibodies with related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobin libraries (Burton, *Proc. Natl. Acad. Sci. 88*, 11120-23, 1991).

Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, *Eur. J. Cancer Prev. 5*, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma & Morrison, 1997, *Nat. Biotechnol. 15*, 159-63. Construction of bivalent, bispecific single-chain antibodies is taught in Mallender & Voss, 1994, *J. Biol. Chem. 269*, 199-206.

A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology (Verhaar *et al.*, 1995, *Int. J. Cancer 61*, 497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth. 165*, 81-91).

Antibodies which specifically bind to CysLT2-like GPCR polypeptides also can be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi *et al.*, *Proc. Natl. Acad. Sci. 86*, 3833-3837, 1989; Winter *et al.*, *Nature 349*, 293-299, 1991).

Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

Antibodies according to the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which a CysLT2-like GPCR polypeptide is bound. The bound antibodies can then be eluted from the column using a buffer with a high salt concentration.

Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences which are complementary to a specific DNA or RNA sequence. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form complexes

and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a cell as described above to decrease the level of CysLT2-like GPCR protein gene products in the cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, or a combination of both. Oligonucleotides can be synthesized manually or by an automated synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. *See* Brown, *Meth. Mol. Biol. 20*, 1-8, 1994; Sonveaux, *Meth. Mol. Biol. 26*, 1-72, 1994; Uhlmann *et al.*, *Chem. Rev. 90*, 543-583, 1990.

Modifications of CysLT2-like GPCR protein gene expression can be obtained by designing antisense oligonucleotides which will form duplexes to the control, 5', or regulatory regions of the CysLT2-like GPCR protein gene. Oligonucleotides derived from the transcription initiation site, *e.g.*, between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described in the literature (*e.g.*, Gee *et al.*, in Huber & Carr, MOLECULAR AND IMMUNOLOGIC APPROACHES, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Precise complementarity is not required for successful complex formation between an antisense oligonucleotide and the complementary sequence of a CysLT2-like GPCR polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a CysLT2-like GPCR polynucleotide, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent CysLT2-like GPCR protein nucleotides, can provide sufficient targeting specificity for CysLT2-like GPCR protein mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular CysLT2-like GPCR polynucleotide sequence.

Antisense oligonucleotides can be modified without affecting their ability to hybridize to a CysLT2-like GPCR polynucleotide. These modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. *See, e.g.*, Agrawal *et al.*, *Trends Biotechnol. 10*, 152-158, 1992; Uhlmann *et al.*, *Chem. Rev. 90*, 543-584, 1990; Uhlmann *et al.*, *Tetrahedron. Lett. 215*, 3539-3542, 1987.

Ribozymes

Ribozymes are RNA molecules with catalytic activity. *See, e.g.*, Cech, *Science 236*, 1532-1539; 1987; Cech, *Ann. Rev. Biochem. 59*, 543-568; 1990, Cech, *Curr. Opin. Struct. Biol. 2*, 605-609; 1992, Couture & Stinchcomb, *Trends Genet. 12*, 510-515, 1996. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (*e.g.*, Haseloff *et al.*, U.S. Patent 5,641,673). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

The coding sequence of a CysLT2-like GPCR polynucleotide, such as the complement of that shown in SEQ ID NO:1, can be used to generate ribozymes which will specifically bind to mRNA transcribed from the CysLT2-like GPCR polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence specific manner have been developed and described in the art (*see* Haseloff *et al. Nature 334*, 585-591, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (see, for example, Gerlach *et al.*, EP 321,201).

Specific ribozyme cleavage sites within a CysLT2-like GPCR protein RNA target can be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate CysLT2-like GPCR protein RNA targets also can be evaluated by testing accessibility to

hybridization with complementary oligonucleotides using ribonuclease protection assays. The nucleotide sequences shown in SEQ ID NOS:1, 3 and their complements provide sources of suitable hybridization region sequences. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related such that upon hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease CysLT2-like GPCR protein expression. Alternatively, if it is desired that the cells stably retain the DNA construct, the construct can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. A ribozyme-encoding DNA construct can include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

As taught in Haseloff *et al.*, U.S. Patent 5,641,673, ribozymes can be engineered so that ribozyme expression will occur in response to factors which induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

Differentially Expressed Genes

Described herein are methods for the identification of genes whose products interact with human CysLT2-like GPCR polypeptide. Such genes may represent genes that are differentially expressed in disorders including, but not limited to, CNS disorders, cardiovascular disorders, osteoporosis, asthma, allergies, and COPD. Further, such

genes may represent genes that are differentially regulated in response to manipulations relevant to the progression or treatment of such diseases. Additionally, such genes may have a temporally modulated expression, increased or decreased at different stages of tissue or organism development. A differentially expressed gene may also have its expression modulated under control versus experimental conditions. In addition, the human CysLT2-like GPCR polypeptide gene or gene product may itself be tested for differential expression.

The degree to which expression differs in a normal versus a diseased state need only be large enough to be visualized via standard characterization techniques such as differential display techniques. Other such standard characterization techniques by which expression differences may be visualized include but are not limited to, quantitative RT (reverse transcriptase), PCR, and Northern analysis.

Identification of Differentially Expressed Genes

To identify differentially expressed genes total RNA or, preferably, mRNA is isolated from tissues of interest. For example, RNA samples are obtained from tissues of experimental subjects and from corresponding tissues of control subjects. Any RNA isolation technique that does not select against the isolation of mRNA may be utilized for the purification of such RNA samples. See, for example, Ausubel *et al.*, ed., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, Inc. New York, 1987-1993. Large numbers of tissue samples may readily be processed using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski, U.S. Patent 4,843,155.

Transcripts within the collected RNA samples that represent RNA produced by differentially expressed genes are identified by methods well known to those of skill in the art. They include, for example, differential screening (Tedder *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 85*, 208-12, 1988), subtractive hybridization (Hedrick *et al.*, *Nature 308*, 149-53; Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 88*, 2825, 1984), and,

preferably, differential display (Liang & Pardee, *Science 257*, 967-71, 1992; U.S. Patent 5,262,311).

The differential expression information may itself suggest relevant methods for the treatment of disorders involving the human CysLT2-like GPCR polypeptide. For example, treatment may include a modulation of expression of the differentially expressed genes and/or the gene encoding the human CysLT2-like GPCR polypeptide. The differential expression information may indicate whether the expression or activity of the differentially expressed gene or gene product or the human CysLT2-like GPCR polypeptide gene or gene product are up-regulated or down-regulated.

Screening Methods

The invention provides assays for screening test compounds which bind to or modulate the activity of a CysLT2-like GPCR polypeptide or a CysLT2-like GPCR polynucleotide. A test compound preferably binds to a CysLT2-like GPCR polypeptide or polynucleotide. More preferably, a test compound decreases or increases a biological effect mediated via human CysLT2-like GPCR protein by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the test compound.

Test Compounds

Test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. The compounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially

addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. *See* Lam, *Anticancer Drug Des. 12*, 145, 1997.

Methods for the synthesis of molecular libraries are well known in the art (*see*, for example, DeWitt *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 90*, 6909, 1993; Erb *et al. Proc. Natl. Acad. Sci. U.S.A. 91*, 11422, 1994; Zuckermann *et al.*, *J. Med. Chem. 37*, 2678, 1994; Cho *et al.*, *Science 261*, 1303, 1993; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2061; Gallop *et al.*, *J. Med. Chem. 37*, 1233, 1994). Libraries of compounds can be presented in solution (*see*, *e.g.*, Houghten, *Biotechniques 13*, 412-421, 1992), or on beads (Lam, *Nature 354*, 82-84, 1991), chips (Fodor, *Nature 364*, 555-556, 1993), bacteria or spores (Ladner, U.S. Patent 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 89*, 1865-1869, 1992), or phage (Scott & Smith, *Science 249*, 386-390, 1990; Devlin, *Science 249*, 404-406, 1990); Cwirla *et al.*, *Proc. Natl. Acad. Sci. 97*, 6378-6382, 1990; Felici, *J. Mol. Biol. 222*, 301-310, 1991; and Ladner, U.S. Patent 5,223,409).

High Throughput Screening

Test compounds can be screened for the ability to bind to CysLT2-like GPCR polypeptides or polynucleotides or to affect CysLT2-like GPCR protein activity or CysLT2-like GPCR protein gene expression using high throughput screening. Using high throughput screening, many discrete compounds can be tested in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. The wells of the microtiter plates typically require assay volumes that range from 50 to 500 µl. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the 96-well format.

Alternatively, "free format assays," or assays that have no physical barrier between samples, can be used. For example, an assay using pigment cells (melanocytes) in a simple homogeneous assay for combinatorial peptide libraries is described by Jayawickreme *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 19,* 1614-18 (1994). The cells are placed under agarose in petri dishes, then beads that carry combinatorial compounds are placed on the surface of the agarose. The combinatorial compounds are partially released the compounds from the beads. Active compounds can be visualized as dark pigment areas because, as the compounds diffuse locally into the gel matrix, the active compounds cause the cells to change colors.

Another example of a free format assay is described by Chelsky, "Strategies for Screening Combinatorial Libraries: Novel and Traditional Approaches," reported at the First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 7-10, 1995). Chelsky placed a simple homogenous enzyme assay for carbonic anhydrase inside an agarose gel such that the enzyme in the gel would cause a color change throughout the gel. Thereafter, beads carrying combinatorial compounds via a photolinker were placed inside the gel and the compounds were partially released by UV-light. Compounds that inhibited the enzyme were observed as local zones of inhibition having less color change.

Yet another example is described by Salmon *et al.*, *Molecular Diversity 2*, 57-63 (1996). In this example, combinatorial libraries were screened for compounds that had cytotoxic effects on cancer cells growing in agar.

Another high throughput screening method is described in Beutel *et al.*, U.S. Patent 5,976,813. In this method, test samples are placed in a porous matrix. One or more assay components are then placed within, on top of, or at the bottom of a matrix such as a gel, a plastic sheet, a filter, or other form of easily manipulated solid support. When samples are introduced to the porous matrix they diffuse sufficiently slowly, such that the assays can be performed without the test samples running together.

Binding Assays

For binding assays, the test compound is preferably a small molecule which binds to and occupies the active site of the CysLT2-like GPCR polypeptide, thereby making the ligand binding site inaccessible to substrate such that normal biological activity is prevented. Examples of such small molecules include, but are not limited to, small peptides or peptide-like molecules. Potential ligands which bind to a polypeptide of the invention include, but are not limited to, the natural ligands of known CysLT2-like GPCR proteins and analogues or derivatives thereof. Natural ligands of GPCRs include adrenomedullin, amylin, calcitonin gene related protein (CGRP), calcitonin, anandamide, serotonin, histamine, adrenalin, noradrenalin, platelet activating factor, thrombin, C5a, bradykinin, and chemokines.

In binding assays, either the test compound or the CysLT2-like GPCR polypeptide can comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or enzymatic label, such as horseradish peroxidase, alkaline phosphatase, or luciferase. Detection of a test compound which is bound to the CysLT2-like GPCR polypeptide can then be accomplished, for example, by direct counting of radioemmission, by scintillation counting, or by determining conversion of an appropriate substrate to a detectable product.

Alternatively, binding of a test compound to a CysLT2-like GPCR polypeptide can be determined without labeling either of the interactants. For example, a microphysiometer can be used to detect binding of a test compound with a CysLT2-like GPCR polypeptide. A microphysiometer (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a test compound and a CysLT2-like GPCR polypeptide (McConnell *et al.*, *Science 257*, 1906-1912, 1992).

Determining the ability of a test compound to bind to a CysLT2-like GPCR polypeptide also can be accomplished using a technology such as real-time Bimolecular Interaction Analysis (BIA) (Sjolander & Urbaniczky, *Anal. Chem. 63*, 2338-2345, 1991, and Szabo *et al.*, *Curr. Opin. Struct. Biol. 5*, 699-705, 1995). BIA is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In yet another aspect of the invention, a CysLT2-like GPCR polypeptide can be used as a "bait protein" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent 5,283,317; Zervos *et al.*, *Cell 72*, 223-232, 1993; Madura *et al.*, *J. Biol. Chem. 268*, 12046-12054, 1993; Bartel *et al.*, *Biotechniques 14*, 920-924, 1993; Iwabuchi *et al.*, *Oncogene 8*, 1693-1696, 1993; and Brent W094/10300), to identify other proteins which bind to or interact with the CysLT2-like GPCR polypeptide and modulate its activity.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. For example, in one construct, polynucleotide encoding a CysLT2-like GPCR polypeptide can be fused to a polynucleotide encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct a DNA sequence that encodes an unidentified protein ("prey" or "sample") can be fused to a polynucleotide that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact *in vivo* to form an protein-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ), which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the

functional transcription factor can be isolated and used to obtain the DNA sequence encoding the protein which interacts with the CysLT2-like GPCR polypeptide.

It may be desirable to immobilize either the CysLT2-like GPCR polypeptide (or polynucleotide) or the test compound to facilitate separation of bound from unbound forms of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the CysLT2-like GPCR polypeptide (or polynucleotide) or the test compound can be bound to a solid support. Suitable solid supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the CysLT2-like GPCR polypeptide (or polynucleotide) or test compound to a solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the polypeptide (or polynucleotide) or test compound and the solid support. Test compounds are preferably bound to the solid support in an array, so that the location of individual test compounds can be tracked. Binding of a test compound to a CysLT2-like GPCR polypeptide (or polynucleotide) can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

In one embodiment, the CysLT2-like GPCR polypeptide is a fusion protein comprising a domain that allows the CysLT2-like GPCR polypeptide to be bound to a solid support. For example, glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and the non-adsorbed CysLT2-like GPCR polypeptide; the mixture is then incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components. Binding of the interactants can be determined either directly or indirectly, as

described above. Alternatively, the complexes can be dissociated from the solid support before binding is determined.

Other techniques for immobilizing proteins or polynucleotides on a solid support also can be used in the screening assays of the invention. For example, either a CysLT2-like GPCR polypeptide (or polynucleotide) or a test compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated CysLT2-like GPCR polypeptides (or polynucleotides) or test compounds can be prepared from biotin-NHS(N-hydroxysuccinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.) and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which specifically bind to a CysLT2-like GPCR polypeptide, polynucleotide, or a test compound, but which do not interfere with a desired binding site, such as the active site of the CysLT2-like GPCR polypeptide, can be derivatized to the wells of the plate. Unbound target or protein can be trapped in the wells by antibody conjugation.

Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies which specifically bind to the CysLT2-like GPCR polypeptide or test compound, enzyme-linked assays which rely on detecting an activity of the CysLT2-like GPCR polypeptide, and SDS gel electrophoresis under non-reducing conditions.

Screening for test compounds which bind to a CysLT2-like GPCR polypeptide or polynucleotide also can be carried out in an intact cell. Any cell which comprises a CysLT2-like GPCR polypeptide or polynucleotide can be used in a cell-based assay system. A CysLT2-like GPCR polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Binding of the test compound to a CysLT2-like GPCR polypeptide or polynucleotide is determined as described above.

Functional Assays

Test compounds can be tested for the ability to increase or decrease a biological effect of a CysLT2-like GPCR polypeptide. Such biological effects can be determined using the functional assays described in the specific examples, below. Functional assays can be carried out after contacting either a purified CysLT2-like GPCR polypeptide, a cell membrane preparation, or an intact cell with a test compound. A test compound which decreases a functional activity of a CysLT2-like GPCR protein by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential agent for decreasing CysLT2-like GPCR protein activity. A test compound which increases CysLT2-like GPCR protein activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential agent for increasing CysLT2-like GPCR protein activity.

One such screening procedure involves the use of melanophores which are transfected to express a CysLT2-like GPCR polypeptide. Such a screening technique is described in WO 92/01810 published Feb. 6, 1992. Thus, for example, such an assay may be employed for screening for a compound which inhibits activation of the receptor polypeptide by contacting the melanophore cells which comprise the receptor with both a receptor ligand and a test compound to be screened. Inhibition of the signal generated by the ligand indicates that a test compound is a potential antagonist for the receptor, *i.e.*, inhibits activation of the receptor. The screen may be employed for identifying a test compound which activates the receptor by contacting such cells with compounds to be screened and determining whether each test compound generates a signal, *i.e.*, activates the receptor.

Other screening techniques include the use of cells which express a human CysLT2-like GPCR polypeptide (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation (*see, e.g., Science*

246, 181-296, 1989). For example, test compounds may be contacted with a cell which expresses a human CysLT2-like GPCR polypeptide and a second messenger response, *e.g.*, signal transduction or pH changes, can be measured to determine whether the test compound activates or inhibits the receptor.

Another such screening technique involves introducing RNA encoding a human CysLT2-like GPCR polypeptide into *Xenopus* oocytes to transiently express the receptor. The transfected oocytes can then be contacted with the receptor ligand and a test compound to be screened, followed by detection of inhibition or activation of a calcium signal in the case of screening for test compounds which are thought to inhibit activation of the receptor.

Another screening technique involves expressing a human CysLT2-like GPCR polypeptide in cells in which the receptor is linked to a phospholipase C or D. Such cells include endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The screening may be accomplished as described above by quantifying the degree of activation of the receptor from changes in the phospholipase activity.

Details of functional assays such as those described above are provided in the specific examples, below.

CysLT2-like GPCR Gene Expression

In another embodiment, test compounds which increase or decrease CysLT2-like GPCR protein gene expression are identified. A CysLT2-like GPCR polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the CysLT2-like GPCR polynucleotide is determined. The level of expression of appropriate mRNA or polypeptide in the presence of the test compound is compared to the level of expression of mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of mRNA or

polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of the mRNA or polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of the mRNA or polypeptide expression.

The level of CysLT2-like GPCR protein mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or polypeptide. Either qualitative or quantitative methods can be used. The presence of polypeptide products of a CysLT2-like GPCR polynucleotide can be determined, for example, using a variety of techniques known in the art, including immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, polypeptide synthesis can be determined *in vivo*, in a cell culture, or in an *in vitro* translation system by detecting incorporation of labeled amino acids into a CysLT2-like GPCR polypeptide.

Such screening can be carried out either in a cell-free assay system or in an intact cell. Any cell which expresses a CysLT2-like GPCR polynucleotide can be used in a cell-based assay system. The CysLT2-like GPCR polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line, such as CHO or human embryonic kidney 293 cells, can be used.

Pharmaceutical Compositions

The invention also provides pharmaceutical compositions which can be administered to a patient to achieve a therapeutic effect. Pharmaceutical compositions of the invention can comprise, for example, a CysLT2-like GPCR polypeptide, CysLT2-like GPCR polynucleotide, antibodies which specifically bind to a CysLT2-like GPCR polypeptide, or mimetics, agonists, antagonists, or inhibitors of a CysLT2-like GPCR polypeptide activity. The compositions can be administered alone or in

combination with at least one other agent, such as stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Pharmaceutical compositions of the invention can be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxy-propylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents can be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which also can contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, *i.e.*, dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers also can be used for delivery. Optionally, the suspension also can contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention can be manufactured in a manner that is known in the art, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. The pharmaceutical composition can be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation can be a lyophilized powder which can contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Maack Publishing Co., Easton, Pa.). After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

Therapeutic Indications and Methods

GPCRs are ubiquitous in the mammalian host and are responsible for many biological functions, including many pathologies. Accordingly, it is desirable to find compounds and drugs which stimulate a GPCR on the one hand and which can inhibit the function of a GPCR on the other hand. For example, compounds which activate a GPCR may be employed for therapeutic purposes, such as the treatment of asthma, Parkinson's disease, acute heart failure, urinary retention, and osteoporosis. In particular, compounds which activate GPCRs are useful in treating various cardiovascular ailments such as caused by the lack of pulmonary blood flow or hypertension. In addition these compounds may also be used in treating various physiological disorders relating to abnormal control of fluid and electrolyte

homeostasis and in diseases associated with abnormal angiotensin-induced aldosterone secretion.

In general, compounds which inhibit activation of a GPCR can be used for a variety of therapeutic purposes, for example, for the treatment of hypotension and/or hypertension, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders including schizophrenia, manic excitement, depression, delirium, dementia or severe mental retardation, dyskinesias, such as Huntington's disease or Tourett's syndrome, among others. Compounds which inhibit GPCRs also are useful in reversing endogenous anorexia and in the control of bulimia.

In particular CysLT2-like GPCR polypeptides can be regulated to treat CNS disorders, cardiovascular disorders, osteoporosis, asthma, allergies, and COPD.

Disorders of the nervous system. CysLT2-like GPCR can be regulated to treat disorders of the nervous system. Disorders of the nervous system which may be treated include brain injuries, cerebrovascular diseases and their consequences, Parkinson's disease, corticobasal degeneration, motor neuron disease, dementia, including ALS, multiple sclerosis, traumatic brain injury, stroke, post-stroke, post-traumatic brain injury, and small-vessel cerebrovascular disease. Dementias, such as Alzheimer's disease, vascular dementia, dementia with Lewy bodies, frontotemporal dementia and Parkinsonism linked to chromosome 17, frontotemporal dementias, including Pick's disease, progressive nuclear palsy, corticobasal degeneration, Huntington's disease, thalamic degeneration, Creutzfeld-Jakob dementia, HIV dementia, schizophrenia with dementia, and Korsakoff's psychosis also can be treated. Similarly, it may be possible to treat cognitive-related disorders, such as mild cognitive impairment, age-associated memory impairment, age-related cognitive decline, vascular cognitive impairment, attention deficit disorders, attention deficit hyperactivity disorders, and memory disturbances in children with learning disabilities, by regulating the activity of CysLT2-like GPCR.

Pain that is associated with nervous system disorders also can be treated by regulating the activity of CysLT2-like GPCR. Pain which can be treated includes that associated with central nervous system disorders, such as multiple sclerosis, spinal cord injury, sciatica, failed back surgery syndrome, traumatic brain injury, epilepsy, Parkinson's disease, post-stroke, and vascular lesions in the brain and spinal cord (*e.g.*, infarct, hemorrhage, vascular malformation). Non-central neuropathic pain includes that associated with post mastectomy pain, reflex sympathetic dystrophy (RSD), trigeminal neuralgia, radioculopathy, post-surgical pain, HIV/AIDS related pain, cancer pain, metabolic neuropathies (*e.g.*, diabetic neuropathy) vasculitic neuropathy (e.g. secondary to connective tissue disease), paraneoplastic polyneuropathy associated, for example, with carcinoma of lung, or leukemia, or lymphoma, or carcinoma of prostate, colon or stomach, and post-herpetic neuralgia and chronic inflammatory pain. Pain associated with cancer and cancer treatment also can be treated, as can headache pain (for example, migraine with aura, migraine without aura, and other migraine disorders), episodic and chronic tension-type headache, tension-type like headache, cluster headache, and chronic paroxysmal hemicrania. By regulation of the CysLT2-like GPCR one can also treat visceral pain as pancreatits, intestinal cystitis, dysmenorrhea, irritable Bowel syndrome, Crohn's disease, biliary colic, ureteral colic, myocardial infarction and pain syndromes of the pelvic cavity, e.g. vulvodynia, orchialgia, urethral syndrome and protatodynia.

Cardiovascular Disorders. Cardiovascular diseases include the following disorders of the heart and the vascular system: congestive heart failure, myocardial infarction, ischemic diseases of the heart, all kinds of atrial and ventricular arrhythmias, hypertensive vascular diseases, and peripheral vascular diseases.

Heart failure is defined as a pathophysiologic state in which an abnormality of cardiac function is responsible for the failure of the heart to pump blood at a rate commensurate with the requirement of the metabolizing tissue. It includes all forms

of pumping failure, such as high-output and low-output, acute and chronic, right-sided or left-sided, systolic or diastolic, independent of the underlying cause.

Myocardial infarction (MI) is generally caused by an abrupt decrease in coronary blood flow that follows a thrombotic occlusion of a coronary artery previously narrowed by arteriosclerosis. MI prophylaxis (primary and secondary prevention) is included, as well as the acute treatment of MI and the prevention of complications.

Ischemic diseases are conditions in which the coronary flow is restricted resulting in a perfusion which is inadequate to meet the myocardial requirement for oxygen. This group of diseases includes stable angina, unstable angina, and asymptomatic ischemia.

Arrhythmias include all forms of atrial and ventricular tachyarrhythmias (atrial tachycardia, atrial flutter, atrial fibrillation, atrio-ventricular reentrant tachycardia, preexcitation syndrome, ventricular tachycardia, ventricular flutter, and ventricular fibrillation), as well as bradycardic forms of arrhythmias.

Vascular diseases include primary as well as all kinds of secondary arterial hypertension (renal, endocrine, neurogenic, others). The disclosed gene and its product may be used as drug targets for the treatment of hypertension as well as for the prevention of all complications. Peripheral vascular diseases are defined as vascular diseases in which arterial and/or venous flow is reduced resulting in an imbalance between blood supply and tissue oxygen demand. It includes chronic peripheral arterial occlusive disease (PAOD), acute arterial thrombosis and embolism, inflammatory vascular disorders, Raynaud's phenomenon, and venous disorders.

Osteoporosis. Osteoporosis is a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk. It is the most common human metabolic

bone disorder. Established osteoporosis includes the presence of fractures. Bone turnover occurs by the action of two major effector cell types within bone: the osteoclast, which is responsible for bone resorption, and the osteoblast, which synthesizes and mineralizes bone matrix. The actions of osteoclasts and osteoblasts are highly co-ordinated. Osteoclast precursors are recruited to the site of turnover; they differentiate and fuse to form mature osteoclasts which then resorb bone. Attached to the bone surface, osteoclasts produce an acidic microenvironment in a tightly defined junction between the specialized osteoclast border membrane and the bone matrix, thus allowing the localized solubilization of bone matrix. This in turn facilitate the proteolysis of demineralized bone collagen. Matrix degradation is thought to release matrix-associated growth factor and cytokines, which recruit osteoblasts in a temporally and spatially controlled fashion. Osteoblasts synthesise and secrete new bone matrix proteins, and subsequently mineralise this new matrix. In the normal skeleton this is a physiological process which does not result in a net change in bone mass. In pathological states, such as osteoporosis, the balance between resorption and formation is altered such that bone loss occurs. See WO 99/45923.

The osteoclast itself is the direct or indirect target of all currently available osteoporosis agents with the possible exception of fluoride. Antiresorptive therapy prevents further bone loss in treated individuals. Osteoblasts are derived from multipotent stem cells which reside in bone marrow and also gives rise to adipocytes, chondrocytes, fibroblasts and muscle cells. Selective enhancement of osteoblast activity is a highly desirable goal for osteoporosis therapy since it would result in an increase in bone mass, rather than a prevention of further bone loss. An effective anabolic therapy would be expected to lead to a significantly greater reduction in fracture risk than currently available treatments.

The agonists or antagonists to the newly discovered polypeptides may act as antiresorptive by directly altering the osteoclast differentiation, osteoclast adhesion to the bone matrix or osteoclast function of degrading the bone matrix. The agonists or

antagonists could indirectly alter the osteoclast function by interfering in the synthesis and/or modification of effector molecules of osteoclast differentiation or function such as cytokines, peptide or steroid hormones, proteases, etc.

The agonists or antagonists to the newly discovered polypeptides may act as anabolics by directly enhancing the osteoblast differentiation and /or its bone matrix forming function. The agonists or antagonists could also indirectly alter the osteoblast function by enhancing the synthesis of growth factors, peptide or steroid hormones or decreasing the synthesis of inhibitory molecules.

The agonists and antagonists may be used to mimic, augment or inhibit the action of the newly discovered polypeptides which may be useful to treat osteoporosis, Paget's disease, degradation of bone implants particularly dental implants.

Asthma and allergies. Allergy is a complex process in which environmental antigens induce clinically adverse reactions. The inducing antigens, called allergens, typically elicit a specific IgE response and, although in most cases the allergens themselves have little or no intrinsic toxicity, they induce pathology when the IgE response in turn elicits an IgE-dependent or T cell-dependent hypersensitivity reaction. Hypersensitivity reactions can be local or systemic and typically occur within minutes of allergen exposure in individuals who have previously been sensitized to an allergen. The hypersensitivity reaction of allergy develops when the allergen is recognized by IgE antibodies bound to specific receptors on the surface of effector cells, such as mast cells, basophils, or eosinophils, which causes the activation of the effector cells and the release of mediators that produce the acute signs and symptoms of the reactions. Allergic diseases include asthma, allergic rhinitis (hay fever), atopic dermatitis, and anaphylaxis.

Asthma is though to arise as a result of interactions between multiple genetic and environmental factors and is characterized by three major features: 1) intermittent and reversible airway obstruction caused by bronchoconstriction, increased mucus

production, and thickening of the walls of the airways that leads to a narrowing of the airways, 2) airway hyperresponsiveness caused by a decreased control of airway caliber, and 3) airway inflammation. Certain cells are critical to the inflammatory reaction of asthma and they include T cells and antigen presenting cells, B cells that produce IgE, and mast cells, basophils, eosinophils, and other cells that bind IgE. These effector cells accumulate at the site of allergic reaction in the airways and release toxic products that contribute to the acute pathology and eventually to the tissue destruction related to the disorder. Other resident cells, such as smooth muscle cells, lung epithelial cells, mucus-producing cells, and nerve cells may also be abnormal in individuals with asthma and may contribute to the pathology. While the airway obstruction of asthma, presenting clinically as an intermittent wheeze and shortness of breath, is generally the most pressing symptom of the disease requiring immediate treatment, the inflammation and tissue destruction associated with the disease can lead to irreversible changes that eventually make asthma a chronic disabling disorder requiring long-term management.

Despite recent important advances in our understanding of the pathophysiology of asthma, the disease appears to be increasing in prevalence and severity (Gergen and Weiss, *Am. Rev. Respir. Dis. 146*, 823-24, 1992). It is estimated that 30-40% of the population suffer with atopic allergy, and 15% of children and 5% of adults in the population suffer from asthma (Gergen and Weiss, 1992). Thus, an enormous burden is placed on our health care resources. However, both diagnosis and treatment of asthma are difficult. The severity of lung tissue inflammation is not easy to measure and the symptoms of the disease are often indistinguishable from those of respiratory infections, chronic respiratory inflammatory disorders, allergic rhinitis, or other respiratory disorders. Often, the inciting allergen cannot be determined, making removal of the causative environmental agent difficult. Current pharmacological treatments suffer their own set of disadvantages. Commonly used therapeutic agents, such as beta agonists, can act as symptom relievers to transiently improve pulmonary function, but do not affect the underlying inflammation. Agents that can reduce the underlying inflammation, such as anti-inflammatory steroids, can have major

drawbacks that range from immunosuppression to bone loss (Goodman and Gilman's THE PHARMACOLOGIC BASIS OF THERAPEUTICS, Seventh Edition, MacMillan Publishing Company, NY, USA, 1985). In addition, many of the present therapies, such as inhaled corticosteroids, are short-lasting, inconvenient to use, and must be used often on a regular basis, in some cases for life, making failure of patients to comply with the treatment a major problem and thereby reducing their effectiveness as a treatment.

Because of the problems associated with conventional therapies, alternative treatment strategies have been evaluated. Glycophorin A (Chu and Sharom, *Cell. Immunol. 145*, 223-39, 1992), cyclosporin (Alexander *et al.*, *Lancet 339*, 324-28, 1992), and a nonapeptide fragment of IL-2 (Zav'yalov *et al.*, *Immunol. Lett. 31*, 285-88, 1992) all inhibit interleukin-2 dependent T lymphocyte proliferation; however, they are known to have many other effects. For example, cyclosporin is used as a immunosuppressant after organ transplantation. While these agents may represent alternatives to steroids in the treatment of asthmatics, they inhibit interleukin-2 dependent T lymphocyte proliferation and potentially critical immune functions associated with homeostasis. Other treatments that block the release or activity of mediators of bronchochonstriction, such as cromones or anti-leukotrienes, have recently been introduced for the treatment of mild asthma, but they are expensive and not effective in all patients and it is unclear whether they have any effect on the chronic changes associated with asthmatic inflammation. What is needed in the art is the identification of a treatment that can act in pathways critical to the development of asthma that both blocks the episodic attacks of the disorder and preferentially dampens the hyperactive allergic immune response without immunocompromising the patient.

Many of the mediators involved in airway smooth muscle contraction and in the chemoattraction of inflammatory cells exert their effects through GPCR binding. Among the mediators of smooth muscle contraction are leukotrienes, platelet-activating factor, endothelin-1, adenosine, and thromboxane A2. Receptor antagonists that block the activation of GPCRs by some of these mediators have been

successfully used as treatments for asthma. Among the chemoattractants of inflammatory cells are the chemokines, such as eotaxin, MCP-4, RANTES, and IL-8. Chemokine receptor antagonists similarly are being developed as treatments for asthma. Sarau *et al.*, *Mol. Pharmacol. 56*, 657-63, 1999; Kitaura *et al.*, *J. Biol. Chem. 271*, 7725-30, 1996; Ligget *et al.*, *Am. J.Respir. Crit. Care Med. 152*, 394-402, 1995; Panettieri *et al.*, *J. Immunol. 154*, 2358-65, 1995; Noveral *et al.*, *Am. J. Physiol. 263*, L317-24, 1992; Honda *et al.*, *Nature 349*, 342-46, 1991.

Activation of some GPCRs may conversely have beneficial effects in asthma. For example, receptor agonists that activate the β1- and β2-adrenergic GPCRs are used therapeutically to relax contracted airway smooth muscle in the treatment of asthma attacks. Thus, regulation of GPCRs in either a positive or negative manner may play an important role in the treatment of asthma.

COPD. Chronic obstructive pulmonary (or airways) disease (COPD) is a condition defined physiologically as airflow obstruction that generally results from a mixture of emphysema and peripheral airway obstruction due to chronic bronchitis (Senior & Shapiro, *Pulmonary Diseases and Disorders*, 3d ed., New York, McGraw-Hill, 1998, pp. 659-681, 1998; Barnes, *Chest 117*, 10S-14S, 2000). Emphysema is characterized by destruction of alveolar walls leading to abnormal enlargement of the air spaces of the lung. Chronic bronchitis is defined clinically as the presence of chronic productive cough for three months in each of two successive years. In COPD, airflow obstruction is usually progressive and is only partially reversible. By far the most important risk factor for development of COPD is cigarette smoking, although the disease does occur in non-smokers.

Chronic inflammation of the airways is a key pathological feature of COPD (Senior & Shapiro, 1998). The inflammatory cell population comprises increased numbers of macrophages, neutrophils, and $CD8^+$ lymphocyes. Inhaled irritants, such as cigarette smoke, activate macrophages which are resident in the respiratory tract, as well as epithelial cells leading to release of chemokines (*e.g.*, interleukin-8) and other

chemotactic factors. These chemotactic factors act to increase the neutrophil/monocyte trafficking from the blood into the lung tissue and airways. Neutrophils and monocytes recruited into the airways can release a variety of potentially damaging mediators such as proteolytic enzymes and reactive oxygen species. Matrix degradation and emphysema, along with airway wall thickening, surfactant dysfunction, and mucus hypersecretion, all are potential sequelae of this inflammatory response that lead to impaired airflow and gas exchange.

This invention further pertains to the use of novel agents identified by the screening assays described above. Accordingly, it is within the scope of this invention to use a test compound identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a modulating agent, an antisense nucleic acid molecule, a specific antibody, ribozyme, or a CysLT2-like GPCR polypeptide binding molecule) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

A reagent which affects CysLT2-like GPCR protein activity can be administered to a human cell, either *in vitro* or *in vivo*, to reduce CysLT2-like GPCR protein activity. The reagent preferably binds to an expression product of a human CysLT2-like GPCR protein gene. If the expression product is a protein, the reagent is preferably an antibody. For treatment of human cells *ex vivo*, an antibody can be added to a preparation of stem cells which have been removed from the body. The cells can then be replaced in the same or another human body, with or without clonal propagation, as is known in the art.

In one embodiment, the reagent is delivered using a liposome. Preferably, the liposome is stable in the animal into which it has been administered for at least about

30 minutes, more preferably for at least about 1 hour, and even more preferably for at least about 24 hours. A liposome comprises a lipid composition that is capable of targeting a reagent, particularly a polynucleotide, to a particular site in an animal, such as a human. Preferably, the lipid composition of the liposome is capable of targeting to a specific organ of an animal, such as the lung, liver, spleen, heart brain, lymph nodes, and skin.

A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents to the cell. Preferably, the transfection efficiency of a liposome is about 0.5 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, more preferably about 1.0 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, and even more preferably about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells. Preferably, a liposome is between about 100 and 500 nm, more preferably between about 150 and 450 nm, and even more preferably between about 200 and 400 nm in diameter.

Suitable liposomes for use in the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Optionally, a liposome comprises a compound capable of targeting the liposome to a tumor cell, such as a tumor cell ligand exposed on the outer surface of the liposome.

Complexing a liposome with a reagent such as an antisense oligonucleotide or ribozyme can be achieved using methods which are standard in the art (see, for example, U.S. Patent 5,705,151). Preferably, from about 0.1 μg to about 10 μg of polynucleotide is combined with about 8 nmol of liposomes, more preferably from about 0.5 μg to about 5 μg of polynucleotides are combined with about 8 nmol

liposomes, and even more preferably about 1.0 μg of polynucleotides is combined with about 8 nmol liposomes.

In another embodiment, antibodies can be delivered to specific tissues *in vivo* using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques are taught in, for example, Findeis *et al. Trends in Biotechnol. 11*, 202-05 (1993); Chiou *et al.*, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.) (1994); Wu & Wu, *J. Biol. Chem. 263*, 621-24 (1988); Wu *et al.*, *J. Biol. Chem. 269*, 542-46 (1994); Zenke *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 87*, 3655-59 (1990); Wu *et al.*, *J. Biol. Chem. 266*, 338-42 (1991).

Determination of a Therapeutically Effective Dose

The determination of a therapeutically effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which increases or decreases CysLT2-like GPCR protein activity relative to the CysLT2-like GPCR protein activity which occurs in the absence of the therapeutically effective dose.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

Therapeutic efficacy and toxicity, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50}.

Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors which can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts can vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

If the reagent is a single-chain antibody, polynucleotides encoding the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads,

protoplast fusion, viral infection, electroporation, "gene gun," and DEAE- or calcium phosphate-mediated transfection.

Effective *in vivo* dosages of an antibody are in the range of about 5 μg to about 50 μg/kg, about 50 μg to about 5 mg/kg, about 100 μg to about 500 μg/kg of patient body weight, and about 200 to about 250 μg/kg of patient body weight. For administration of polynucleotides encoding single-chain antibodies, effective *in vivo* dosages are in the range of about 100 ng to about 200 ng, 500 ng to about 50 mg, about 1 μg to about 2 mg, about 5 μg to about 500 μg, and about 20 μg to about 100 μg of DNA.

If the expression product is mRNA, the reagent is preferably an antisense oligonucleotide or a ribozyme. Polynucleotides which express antisense oligonucleotides or ribozymes can be introduced into cells by a variety of methods, as described above.

Preferably, a reagent reduces expression of a CysLT2-like GPCR protein gene or the activity of a CysLT2-like GPCR polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the level of expression of a CysLT2-like GPCR protein gene or the activity of a CysLT2-like GPCR polypeptide can be assessed using methods well known in the art, such as hybridization of nucleotide probes to CysLT2-like GPCR protein-specific mRNA, quantitative RT-PCR, immunologic detection of a CysLT2-like GPCR polypeptide, or measurement of CysLT2-like GPCR protein activity.

In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act synergistically to effect the

treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Any of the therapeutic methods described above can be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

Diagnostic Methods

GPCRs also can be used in diagnostic assays for detecting diseases and abnormalities or susceptibility to diseases and abnormalities related to the presence of mutations in the nucleic acid sequences which encode a GPCR. Such diseases, by way of example, are related to cell transformation, such as tumors and cancers, and various cardiovascular disorders, including hypertension and hypotension, as well as diseases arising from abnormal blood flow, abnormal angiotensin-induced aldosterone secretion, and other abnormal control of fluid and electrolyte homeostasis.

Differences can be determined between the cDNA or genomic sequence encoding a GPCR in individuals afflicted with a disease and in normal individuals. If a mutation is observed in some or all of the afflicted individuals but not in normal individuals, then the mutation is likely to be the causative agent of the disease.

Sequence differences between a reference gene and a gene having mutations can be revealed by the direct DNA sequencing method. In addition, cloned DNA segments can be employed as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequencing primer can be used with a double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures using radiolabeled nucleotides or by automatic sequencing procedures using fluorescent tags.

Genetic testing based on DNA sequence differences can be carried out by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized, for example, by high resolution gel electrophoresis. DNA fragments of different sequences can be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (*see, e.g.*, Myers *et al.*, *Science 230*, 1242, 1985). Sequence changes at specific locations can also be revealed by nuclease protection assays, such as RNase and S 1 protection or the chemical cleavage method (*e.g.*, Cotton *et al.*, *Proc. Natl. Acad. Sci. USA 85*, 4397-4401, 1985). Thus, the detection of a specific DNA sequence can be performed by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes and Southern blotting of genomic DNA. In addition to direct methods such as gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

Altered levels of a GPCR also can be detected in various tissues. Assays used to detect levels of the receptor polypeptides in a body sample, such as blood or a tissue biopsy, derived from a host are well known to those of skill in the art and include radioimmunoassays, competitive binding assays, Western blot analysis, and ELISA assays.

All patents and patent applications cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

Detection of CysLT2-like GPCR activity

The polynucleotide of SEQ ID NO: 1 is inserted into the expression vector pCEV4 and the expression vector pCEV4-CysLT2-like GPCR polypeptide obtained is transfected into human embryonic kidney 293 cells. The cells are scraped from a culture flask into 5 ml of Tris HCl, 5 mM EDTA, pH 7.5, and lysed by sonication. Cell lysates are centrifuged at 1000 rpm for 5 minutes at 4 °C. The supernatant is centrifuged at 30,000 x g for 20 minutes at 4 °C. The pellet is suspended in binding buffer containing 50 mM Tris HCl, 5 mM $MgSO_4$, 1 mM EDTA, 100 mM NaCl, pH 7.5, supplemented with 0.1 % BSA, 2 µg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 µg/ml phosphoramidon. Optimal membrane suspension dilutions, defined as the protein concentration required to bind less than 10 % of an added radioligand, i.e. ^{125}I-labeled CysLT2, are added to 96-well polypropylene microtiter plates containing ligand, non-labeled peptides, and binding buffer to a final volume of 250 µl.

In equilibrium saturation binding assays, membrane preparations are incubated in the presence of increasing concentrations (0.1 nM to 4 nM) of ^{125}I ligand.

Binding reaction mixtures are incubated for one hour at 30 °C. The reaction is stopped by filtration through GF/B filters treated with 0.5% polyethyleneimine, using a cell harvester. Radioactivity is measured by scintillation counting, and data are analyzed by a computerized non-linear regression program. Non-specific binding is defined as the amount of radioactivity remaining after incubation of membrane protein in the presence of 100 nM of unlabeled peptide. Protein concentration is measured by the Bradford method using Bio-Rad Reagent, with bovine serum albumin as a standard. The CysLT2-like GPCR activity of the polypeptide comprising the amino acid sequence of SEQ ID NO: 2 is demonstrated.

EXAMPLE 2

Radioligand binding assays

Human embryonic kidney 293 cells transfected with a polynucleotide which expresses human CysLT2-like GPCR protein are scraped from a culture flask into 5 ml of Tris HCl, 5 mM EDTA, pH 7.5, and lysed by sonication. Cell lysates are centrifuged at 1000 rpm for 5 minutes at 4 °C. The supernatant is centrifuged at 30,000 x g for 20 minutes at 4 °C. The pellet is suspended in binding buffer containing 50 mM Tris HCl, 5 mM $MgSO_4$, 1 mM EDTA, 100 mM NaCl, pH 7.5, supplemented with 0.1 % BSA, 2 μg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 μg/ml phosphoramidon. Optimal membrane suspension dilutions, defined as the protein concentration required to bind less than 10 % of the added radioligand, i.e. CysLT2, are added to 96-well polypropylene microtiter plates containing ^{125}I-labeled ligand or test compound, non-labeled peptides, and binding buffer to a final volume of 250 μl.

In equilibrium saturation binding assays, membrane preparations are incubated in the presence of increasing concentrations (0.1 nM to 4 nM) of ^{125}I-labeled ligand or test compound (specific activity 2200 Ci/mmol). The binding affinities of different test compounds are determined in equilibrium competition binding assays, using 0.1 nM ^{125}I-peptide in the presence of twelve different concentrations of each test compound.

Binding reaction mixtures are incubated for one hour at 30 °C. The reaction is stopped by filtration through GF/B filters treated with 0.5% polyethyleneimine, using a cell harvester. Radioactivity is measured by scintillation counting, and data are analyzed by a computerized non-linear regression program.

Non-specific binding is defined as the amount of radioactivity remaining after incubation of membrane protein in the presence of 100 nM of unlabeled peptide. Protein concentration is measured by the Bradford method using Bio-Rad Reagent,

with bovine serum albumin as a standard. A test compound which increases the radioactivity of membrane protein by at least 15% relative to radioactivity of membrane protein which was not incubated with a test compound is identified as a compound which binds to a human CysLT2-like GPCR polypeptide.

EXAMPLE 3

Effect of a test compound on human CysLT2-like GPCR protein-mediated cyclic AMP formation

Receptor-mediated inhibition of cAMP formation can be assayed in host cells which express human CysLT2-like GPCR protein. Cells are plated in 96-well plates and incubated in Dulbecco□s phosphate buffered saline (PBS) supplemented with 10 mM HEPES, 5 mM theophylline, 2 μg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 μg/ml phosphoramidon for 20 minutes at 37 °C in 5% CO_2. A test compound is added and incubated for an additional 10 minutes at 37 °C. The medium is aspirated, and the reaction is stopped by the addition of 100 mM HCl. The plates are stored at 4 °C for 15 minutes. cAMP content in the stopping solution is measured by radioimmunoassay.

Radioactivity is quantified using a gamma counter equipped with data reduction software. A test compound which decreases radioactivity of the contents of a well relative to radioactivity of the contents of a well in the absence of the test compound is identified as a potential inhibitor of cAMP formation. A test compound which increases radioactivity of the contents of a well relative to radioactivity of the contents of a well in the absence of the test compound is identified as a potential enhancer of cAMP formation.

EXAMPLE 4

Effect of a test compound on the mobilization of intracellular calcium

Intracellular free calcium concentration can be measured by microspectrofluorometry using the fluorescent indicator dye Fura-2/AM (Bush *et al.*, *J. Neurochem. 57*, 562-74, 1991). Stably transfected cells are seeded onto a 35 mm culture dish containing a glass coverslip insert. Cells are washed with HBS , incubated with a test compound, and loaded with 100 µl of Fura-2/AM (10 µM) for 20-40 minutes. After washing with HBS to remove the Fura-2/AM solution, cells are equilibrated in HBS for 10-20 minutes. Cells are then visualized under the 40X objective of a Leitz Fluovert FS microscope.

Fluorescence emission is determined at 510 nM, with excitation wavelengths alternating between 340 nM and 380 nM. Raw fluorescence data are converted to calcium concentrations using standard calcium concentration curves and software analysis techniques. A test compound which increases the fluorescence by at least 15% relative to fluorescence in the absence of a test compound is identified as a compound which mobilizes intracellular calcium.

EXAMPLE 5

Effect of a test compound on phosphoinositide metabolism

Cells which stably express human CysLT2-like GPCR protein cDNA are plated in 96-well plates and grown to confluence. The day before the assay, the growth medium is changed to 100 µl of medium containing 1% serum and 0.5 µCi ^{3}H-myinositol. The plates are incubated overnight in a CO_2 incubator (5% CO_2 at 37 °C). Immediately before the assay, the medium is removed and replaced by 200 µl of PBS containing 10 mM LiCl, and the cells are equilibrated with the new medium for 20 minutes. During this interval, cells also are equilibrated with antagonist, added as a 10 µl aliquot of a 20-fold concentrated solution in PBS.

The ^{3}H-inositol phosphate accumulation from inositol phospholipid metabolism is started by adding 10 µl of a solution containing a test compound. To the first well 10 µl are added to measure basal accumulation. Eleven different concentrations of test compound are assayed in the following 11 wells of each plate row. All assays are performed in duplicate by repeating the same additions in two consecutive plate rows.

The plates are incubated in a CO_2 incubator for one hour. The reaction is terminated by adding 15 µl of 50% v/v trichloroacetic acid (TCA), followed by a 40 minute incubation at 4 °C. After neutralizing TCA with 40 µl of 1 M Tris, the content of the wells is transferred to a Multiscreen HV filter plate (Millipore) containing Dowex AG1-X8 (200-400 mesh, formate form). The filter plates are prepared by adding 200 µl of Dowex AG1-X8 suspension (50% v/v, water:resin) to each well. The filter plates are placed on a vacuum manifold to wash or elute the resin bed. Each well is washed 2 times with 200 µl of water, followed by 2 x 200 µl of 5 mM sodium tetraborate/60 mM ammonium formate.

The ^{3}H-IPs are eluted into empty 96-well plates with 200 µl of 1.2 M ammonium formate/0.1 formic acid. The content of the wells is added to 3 ml of scintillation cocktail, and radioactivity is determined by liquid scintillation counting.

EXAMPLE 6

Receptor Binding Methods

Standard Binding Assays. Binding assays are carried out in a binding buffer containing 50 mM HEPES, pH 7.4, 0.5% BSA, and 5 mM $MgCl_2$. The standard assay for radioligand (*e.g.*, ^{125}I- test compound) binding to membrane fragments comprising CysLT2-like GPCR polypeptides is carried out as follows in 96 well microtiter plates (*e.g.*, Dynatech Immulon II Removawell plates). Radioligand is diluted in binding buffer+ PMSF/Baci to the desired cpm per 50 µl, then 50 µl

aliquots are added to the wells. For non-specific binding samples, 5 µl of 40 µM cold ligand also is added per well. Binding is initiated by adding 150 µl per well of membrane diluted to the desired concentration (10-30 µg membrane protein/well) in binding buffer+ PMSF/Baci. Plates are then covered with Linbro mylar plate sealers (Flow Labs) and placed on a Dynatech Microshaker II. Binding is allowed to proceed at room temperature for 1-2 hours and is stopped by centrifuging the plate for 15 minutes at 2,000 x g. The supernatants are decanted, and the membrane pellets are washed once by addition of 200 µl of ice cold binding buffer, brief shaking, and recentrifugation. The individual wells are placed in 12 x 75 mm tubes and counted in an LKB Gammamaster counter (78% efficiency). Specific binding by this method is identical to that measured when free ligand is removed by rapid (3-5 seconds) filtration and washing on polyethyleneimine-coated glass fiber filters.

Three variations of the standard binding assay are also used.

1. Competitive radioligand binding assays with a concentration range of cold ligand vs. ^{125}I-labeled ligand are carried out as described above with one modification. All dilutions of ligands being assayed are made in 40X PMSF/Baci to a concentration 40X the final concentration in the assay. Samples of peptide (5 µl each) are then added per microtiter well. Membranes and radioligand are diluted in binding buffer without protease inhibitors. Radioligand is added and mixed with cold ligand, and then binding is initiated by addition of membranes.

2. Chemical cross-linking of radioligand with receptor is done after a binding step identical to the standard assay. However, the wash step is done with binding buffer minus BSA to reduce the possibility of non-specific cross-linking of radioligand with BSA. The cross-linking step is carried out as described below.

3. Larger scale binding assays to obtain membrane pellets for studies on solubilization of receptor:ligand complex and for receptor purification are also carried out. These are identical to the standard assays except that (a) binding is carried out in polypropylene tubes in volumes from 1-250 ml, (b) concentration of membrane protein is always 0.5 mg/ml, and (c) for receptor purification, BSA concentration in the binding buffer is reduced to 0.25%, and the wash step is done with binding buffer without BSA, which reduces BSA contamination of the purified receptor.

EXAMPLE 7

Chemical Cross-Linking of Radioligand to Receptor

After a radioligand binding step as described above, membrane pellets are resuspended in 200 µl per microtiter plate well of ice-cold binding buffer without BSA. Then 5 µl per well of 4 mM N-5-azido-2-nitrobenzoyloxysuccinimide (ANB-NOS, Pierce) in DMSO is added and mixed. The samples are held on ice and UV-irradiated for 10 minutes with a Mineralight R-52G lamp (UVP Inc., San Gabriel, Calif.) at a distance of 5-10 cm. Then the samples are transferred to Eppendorf microfuge tubes, the membranes pelleted by centrifugation, supernatants removed, and membranes solubilized in Laemmli SDS sample buffer for polyacrylamide gel electrophoresis (PAGE). PAGE is carried out as described below. Radiolabeled proteins are visualized by autoradiography of the dried gels with Kodak XAR film and Dupont image intensifier screens.

EXAMPLE 8

Membrane Solubilization

Membrane solubilization is carried out in buffer containing 25 mM Tris , pH 8, 10% glycerol (w/v) and 0.2 mM $CaCl_2$ (solubilization buffer). The highly soluble detergents including Triton X-100, deoxycholate, deoxycholate:lysolecithin, CHAPS, and zwittergent are made up in solubilization buffer at 10% concentrations and stored

as frozen aliquots. Lysolecithin is made up fresh because of insolubility upon freeze-thawing and digitonin is made fresh at lower concentrations due to its more limited solubility.

To solubilize membranes, washed pellets after the binding step are resuspended free of visible particles by pipetting and vortexing in solubilization buffer at 100,000 x g for 30 minutes. The supernatants are removed and held on ice and the pellets are discarded.

EXAMPLE 9

Assay of Solubilized Receptors

After binding of ^{125}I ligands and solubilization of the membranes with detergent, the intact R:L complex can be assayed by four different methods. All are carried out on ice or in a cold room at 4-10 °C.).

1. Column chromatography (Knuhtsen *et al.*, *Biochem. J. 254*, 641-647, 1988). Sephadex G-50 columns (8 x 250 mm) are equilibrated with solubilization buffer containing detergent at the concentration used to solubilize membranes and 1 mg/ml bovine serum albumin. Samples of solubilized membranes (0.2-0.5 ml) are applied to the columns and eluted at a flow rate of about 0.7 ml/minute. Samples (0.18 ml) are collected. Radioactivity is determined in a gamma counter. Void volumes of the columns are determined by the elution volume of blue dextran. Radioactivity eluting in the void volume is considered bound to protein. Radioactivity eluting later, at the same volume as free ^{125}I ligands, is considered non-bound.

2. Polyethyleneglycol precipitation (Cuatrecasas, *Proc. Natl. Acad. Sci. USA 69*, 318-322, 1972). For a 100 µl sample of solubilized membranes in a 12 x 75 mm polypropylene tube, 0.5 ml of 1% (w/v) bovine gamma globulin (Sigma) in 0.1 M sodium phosphate buffer is added, followed by 0.5 ml of

25% (w/v) polyethyleneglycol (Sigma) and mixing. The mixture is held on ice for 15 minutes. Then 3 ml of 0.1 M sodium phosphate, pH 7.4, is added per sample. The samples are rapidly (1-3 seconds) filtered over Whatman GF/B glass fiber filters and washed with 4 ml of the phosphate buffer. PEG-precipitated receptor : ^{125}I-ligand complex is determined by gamma counting of the filters.

3. GFB/PEI filter binding (Bruns *et al.*, *Analytical Biochem. 132*, 74-81, 1983). Whatman GF/B glass fiber filters are soaked in 0.3% polyethyleneimine (PEI, Sigma) for 3 hours. Samples of solubilized membranes (25-100 µl) are replaced in 12 x 75 mm polypropylene tubes. Then 4 ml of solubilization buffer without detergent is added per sample and the samples are immediately filtered through the GFB/PEI filters (1-3 seconds) and washed with 4 ml of solubilization buffer. CPM of receptor : ^{125}I-ligand complex adsorbed to filters are determined by gamma counting.

4. Charcoal/Dextran (Paul and Said, *Peptides 7[Suppl. 1]*,147-149, 1986). Dextran T70 (0.5 g, Pharmacia) is dissolved in 1 liter of water, then 5 g of activated charcoal (Norit A, alkaline; Fisher Scientific) is added. The suspension is stirred for 10 minutes at room temperature and then stored at 4 °C. until use. To measure R:L complex, 4 parts by volume of charcoal/dextran suspension are added to 1 part by volume of solubilized membrane. The samples are mixed and held on ice for 2 minutes and then centrifuged for 2 minutes at 11,000 x g in a Beckman microfuge. Free radioligand is adsorbed charcoal/dextran and is discarded with the pellet. Receptor : ^{125}I-ligand complexes remain in the supernatant and are determined by gamma counting.

EXAMPLE 10

Receptor Purification

Binding of biotinyl-receptor to GH_4 C1 membranes is carried out as described above. Incubations are for 1 hour at room temperature. In the standard purification protocol, the binding incubations contain 10 nM Bio-S29. ^{125}I ligand is added as a tracer at levels of 5,000-100,000 cpm per mg of membrane protein. Control incubations contain 10 μM cold ligand to saturate the receptor with non-biotinylated ligand.

Solubilization of receptor:ligand complex also is carried out as described above, with 0.15% deoxycholate:lysolecithin in solubilization buffer containing 0.2 mM $MgCl_2$, to obtain 100,000 x g supernatants containing solubilized R:L complex.

Immobilized streptavidin (streptavidin cross-linked to 6% beaded agarose, Pierce Chemical Co.; "SA-agarose") is washed in solubilization buffer and added to the solubilized membranes as 1/30 of the final volume. This mixture is incubated with constant stirring by end-over-end rotation for 4-5 hours at 4-10 °C. Then the mixture is applied to a column and the non-bound material is washed through. Binding of radioligand to SA-agarose is determined by comparing cpm in the 100,000 x g supernatant with that in the column effluent after adsorption to SA-agarose. Finally, the column is washed with 12-15 column volumes of solubilization buffer+0.15% deoxycholate:lysolecithin +1/500 (vol/vol) 100 x 4pase.

The streptavidin column is eluted with solubilization buffer+0.1 mM EDTA+0.1 mM EGTA+0.1 mM GTP-gamma-S (Sigma)+0.15% (wt/vol) deoxycholate:lysolecithin +1/1000 (vol/vol) 100.times.4pase. First, one column volume of elution buffer is passed through the column and flow is stopped for 20-30 minutes. Then 3-4 more column volumes of elution buffer are passed through. All the eluates are pooled.

Eluates from the streptavidin column are incubated overnight (12-15 hours) with immobilized wheat germ agglutinin (WGA agarose, Vector Labs) to adsorb the

receptor via interaction of covalently bound carbohydrate with the WGA lectin. The ratio (vol/vol) of WGA-agarose to streptavidin column eluate is generally 1:400. A range from 1:1000 to 1:200 also can be used. After the binding step, the resin is pelleted by centrifugation, the supernatant is removed and saved, and the resin is washed 3 times (about 2 minutes each) in buffer containing 50 mM HEPES, pH 8, 5 mM $MgCl_2$, and 0.15% deoxycholate:lysolecithin. To elute the WGA-bound receptor, the resin is extracted three times by repeated mixing (vortex mixer on low speed) over a 15-30 minute period on ice, with 3 resin columns each time, of 10 mM N-N'-N"-triacetylchitotriose in the same HEPES buffer used to wash the resin. After each elution step, the resin is centrifuged down and the supernatant is carefully removed, free of WGA-agarose pellets. The three, pooled eluates contain the final, purified receptor. The material non-bound to WGA contain G protein subunits specifically eluted from the streptavidin column, as well as non-specific contaminants. All these fractions are stored frozen at -90 °C.

EXAMPLE 11

Identification of test compounds that bind to CysLT2-like GPCR polypeptides

Purified CysLT2-like GPCR polypeptides comprising a glutathione-S-transferase protein and absorbed onto glutathione-derivatized wells of 96-well microtiter plates are contacted with test compounds from a small molecule library at pH 7.0 in a physiological buffer solution. CysLT2-like GPCR polypeptides comprise an amino acid sequence shown in SEQ ID NO:2. The test compounds comprise a fluorescent tag. The samples are incubated for 5 minutes to one hour. Control samples are incubated in the absence of a test compound.

The buffer solution containing the test compounds is washed from the wells. Binding of a test compound to a CysLT2-like GPCR polypeptide is detected by fluorescence measurements of the contents of the wells. A test compound which increases the fluorescence in a well by at least 15% relative to fluorescence of a well

in which a test compound was not incubated is identified as a compound which binds to a CysLT2-like GPCR polypeptide.

EXAMPLE 12

Identification of a test compound which decreases CysLT2-like GPCR protein gene expression

A test compound is administered to a culture of human gastric cells and incubated at 37 °C for 10 to 45 minutes. A culture of the same type of cells incubated for the same time without the test compound provides a negative control.

RNA is isolated from the two cultures as described in Chirgwin *et al.*, *Biochem. 18*, 5294-99, 1979). Northern blots are prepared using 20 to 30 µg total RNA and hybridized with a ^{32}P-labeled CysLT2-like GPCR protein-specific probe at 65 °C in Express-hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides selected from SEQ ID NO:1. A test compound which decreases the CysLT2-like GPCR protein-specific signal relative to the signal obtained in the absence of the test compound is identified as an inhibitor of CysLT2-like GPCR protein gene expression.

EXAMPLE 13

Treatment of asthma with a reagent which specifically binds to a CysLT2-like GPCR protein gene product

Synthesis of antisense CysLT2-like GPCR oligonucleotides comprising at least 11 contiguous nucleotides selected from SEQ ID NO:1 is performed on a Pharmacia Gene Assembler series synthesizer using the phosphoramidite procedure (Uhlmann *et al.*, *Chem. Rev. 90*, 534-83, 1990). Following assembly and deprotection, oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate-buffered saline (PBS) at the desired concentration. Purity of these oligonucleotides is tested by capillary gel electrophoreses and ion exchange HPLC.

Endotoxin levels in the oligonucleotide preparation are determined using the Luminous Amebocyte Assay (Bang, *Biol. Bull. (Woods Hole, Mass.) 105*, 361-362, 1953).

The antisense oligonucleotides are administered intrabronchially to a patient with asthma. The severity of the patient's asthma is lessened.

EXAMPLE 14

Tissue-specific expression of CysLT2-like GPCR

As a first step to establishing a role for CysLT2-like GPCR in the pathogenesis of COPD, expression profiling of the gene was done using real-time quantitative PCR with RNA samples from human respiratory tissues and inflammatory cells relevant to COPD. The panel consisted of total RNA samples lung (adult and fetal), trachea, freshly isolated alveolar type II cells, cultured human bronchial epithelial cells, cultured small airway epithelial cells, cultured bronchial sooth muscle cells, cultured H441 cells (Clara-like), freshly isolated neutrophils and monocytes, and cultured monocytes (macrophage-like). Expression of CysLT2-like GPCR also was evaluated in a range of human tissues using total RNA panels obtained from Clontech Laboratories, UK, Ltd.. The tissues were adrenal gland, bone marrow, brain, colon, heart, kidney, liver, lung, mammary gland, pancreas, prostate, salivary gland, skeletal muscle, small intesting, spleen, stomach, testis, thymus, trachea, thyroid, and uterus.a development of the kinetic analysis of PCR first described in Higuchi *et al.*, *BioTechnology 10*, 413-17, 1992, and Higuchi *et al.*, *BioTechnology 11*, 1026-30, 1993. The principle is that at any given cycle within the exponential phase of PCR, the amount of product is proportional to the initial number of template copies.

PCR amplification is performed in the presence of an oligonucleotide probe (TaqMan probe) that is complementary to the target sequence and labeled with a fluorescent reporter dye and a quencher dye. During the extension phase of PCR, the probe is cleaved by the 5'-3' endonuclease activity of Taq DNA polymerase,

releasing the fluorophore from the effect of the quenching dye (Holland *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 88*, 7276-80, 1991). Because the fluorescence emission increases in direct proportion to the amount of the specific amplified product, the exponential growth phase of PCR product can be detected and used to determine the initial template concentration (Heid *et al.*, *Genome Res. 6*, 986-94, 1996, and Gibson *et al.*, *Genome Res. 6*, 995-1001, 1996).

Real-time quantitative PCR was done using an ABI Prism 7700 Sequence Detector. The C_T value generated for each reaciton was used to determine the initial template concentration (copy number) by interpolation from a universal standard curve. The level of expression of the target gene in each sample was calculated relative to the sample with the lowest expression of the gene.

RNA extraction and cDNA preparation. Total RNA from each of the respiratory tissues and inflammatory cell types listed above were isolated using Qiagen's RNeasy system according to the manufacturer's protocol (Crawley, West Sussex, UK). The concentration of purified RNA was determined using a RiboGreen RNA quantitation kit (Molecular Probes Europe, The Netherlands). For the preparation of cDNA, 1 μg of total RNA was reverse transcribed in a final volume of 20 μl, using 200 U of SUPERSCRIPT™ RNase H⁻ Reverse Transcriptase (Life Technologies, Paisley, UK), 10 mM dithiothreitol, 0.5 mM of each dNTP and 5 μM random hexamers (Applied Biosystems, Warrington, Cheshire, UK) according to the manufacturer's protocol.

TaqMan quantitative analysis. Specific primers and probe were designed according to the recommendations of PE Applied Biosystems; a FAM (6-carboxy-fluorescein)-labeled probe was used. Quantification PCR was performed with 5 ng of reverse transcribed RNA from each sample. Each determination was done in duplicate.

The assay reaction mix was as follows: 1X final TaqMan Universal PCR Master Mix (from 2X stock) (PE Applied Biosystems, CA); 900 nM forward primer; 900 nM reverse primer; 200 nM probe; 5 ng cDNA; and water to 25 µl.

Each of the following steps were carried out once: pre PCR, 2 minutes at 50 C, and 10 minutes at 95 C. The following steps are carried out 40 times: denaturation, 15 seconds at 95 C, annealing/extension, 1 minute at 60 C.

All experiments were performed using an ABI Prism 7700 Sequence Detector (PE Applied Biosystems, CA). At the end of the run, fluorescence data acquired during PCR were processed as described in the ABI Prism 7700 user's manual to achieve better background subtraction as well as signal linearity with the starting target quantity.

Tables 1 and 2 show the results of expression profiling for CysLT2-like GPCR using the indicated cell and tissue samples. For Table 1, the cells are defined as follows: HBEC, cultured human bronchial epithelial cells; H441, a Clara-like cell line; SAE, cultured small airway epithelial cells; SMC, cultured airway smooth muscle cells; AII, freshly isolated human alveolar type II cells; Neut, freshly isolated circulating neutrophils; Mono, freshly isolated monocytes; and CM, cultured monocytes. Other letters identify the donor. The results are shown graphically in FIGS. 5 and 6. Relative low expression is detected in the fetal and adult brain compared to high expression in heart, lung, colon, small intestine and placenta. Even low expression in the CNS, specific expression of the CysLT2-like GPCR indicates the possibility to treat various disorders of the nervous system. The expression of CysLT2-like GPCR in the specific nervous system tissue is relatative low but ubiquitous through out the brain and spinal cord. The highest expression was detected in the peripheral nervous system in the dorsal root ganglia. This expression of the CysLT2-like GPCR in central as well as in peripheral nervous system tissue indicates the possibility to treat various disorders of the nervous system.

EXAMPLE 15

Expression of recombinant human CysLT2-like GPCR

The *Pichia pastoris* expression vector pPICZB (Invitrogen, San Diego, CA) is used to produce large quantities of recombinant human CysLT2-like GPCR polypeptides in yeast. The CysLT2-like GPCR-encoding DNA sequence is derived from SEQ ID NO:1. Before insertion into vector pPICZB, the DNA sequence is modified by well known methods in such a way that it contains at its 5'-end an initiation codon and at its 3'-end an enterokinase cleavage site, a His6 reporter tag and a termination codon. Moreover, at both termini recognition sequences for restriction endonucleases are added and after digestion of the multiple cloning site of pPICZ B with the corresponding restriction enzymes the modified DNA sequence is ligated into pPICZB. This expression vector is designed for inducible expression in *Pichia pastoris*, driven by a yeast promoter. The resulting pPICZ/md-His6 vector is used to transform the yeast.

The yeast is cultivated under usual conditions in 5 liter shake flasks and the recombinantly produced protein isolated from the culture by affinity chromatography (Ni-NTA-Resin) in the presence of 8 M urea. The bound polypeptide is eluted with buffer, pH 3.5, and neutralized. Separation of the polypeptide from the His6 reporter tag is accomplished by site-specific proteolysis using enterokinase (Invitrogen, San Diego, CA) according to manufacturer's instructions. Purified human CysLT2-like GPCR polypeptide is obtained.

EXAMPLE 16

Quantitative Expression Profiling of CysLT2-like GPCR

Expression profiling is based on a quantitative polymerase chain reaction (PCR) analysis, also called kinetic analysis, first described in Higuchi et al., 1992 and Higuchi et al., 1993. The principle is that at any given cycle within the exponential phase of PCR, the amount of product is proportional to the initial number of template

copies. Using this technique, the expression levels of particular genes, which are transcribed from the chromosomes as messenger RNA (mRNA), are measured by first making a DNA copy (cDNA) of the mRNA, and then performing quantitative PCR on the cDNA, a method called quantitative reverse transcription-polymerase chain reaction (quantitative RT-PCR).

Quantitative RT-PCR analysis of RNA from different human tissues was performed to investigate the tissue distribution of CysLT2-like GPCR mRNA. 25 .mu.g of total RNA from various tissues (Human Total RNA Panel I-V, Clontech Laboratories, Palo Alto, CA, USA) was used as a template to synthsize first-strand cDNA using the SUPERSCRIPT™ First-Strand Synthesis System for RT-PCR (Life Technologies, Rockville , MD, USA). First-strand cDNA synthesis was carried out according to the manufacturer's protocol using oligo (dT) to hybridize to the 3' poly A tails of mRNA and prime the synthesis reaction. 10 ng of the first-strand cDNA was then used as template in a polymerase chain reaction. The polymerase chain reaction was performed in a LightCycler (Roche Molecular Biochemicals, Indianapolis, IN, USA), in the presence of the DNA-binding fluorescent dye SYBR Green I which binds to the minor groove of the DNA double helix, produced only when double-stranded DNA is successfully synthesized in the reaction (Morrison et al., 1998). Upon binding to double-stranded DNA, SYBR Green I emits light that can be quantitatively measured by the LightCycler machine. The polymerase chain reaction was carried out using oligonucleotide primers AA254664-L2 (TGCGTTTCCTGGCAATGGTTCA) and AA254664-R2 (GCAGCCCACCACCAAGGCAATA) and measurements of the intensity of emitted light were taken following each cycle of the reaction when the reaction had reached a temperature of 80 degrees C. Intensities of emitted light were converted into copy numbers of the gene transcript per nanogram of template cDNA by comparison with simultaneously reacted standards of known concentration.

To correct for differences in mRNA transcription levels per cell in the various tissue types, a normalization procedure was performed using similarly calculated

expression levels in the various tissues of five different housekeeping genes: glyceraldehyde-3-phosphatase (G3PDH), hypoxanthine guanine phophoribosyl transferase (HPRT), beta-actin, porphobilinogen deaminase (PBGD), and beta-2-microglobulin. The level of housekeeping gene expression is considered to be relatively constant for all tissues (Adams et al., 1993, Adams et al., 1995, Liew et al., 1994) and therefore can be used as a gauge to approximate relative numbers of cells per .mu.g of total RNA used in the cDNA synthesis step. Except for the use of a slightly different set of housekeeping genes and the use of the LightCycler system to measure expression levels, the normalization procedure was essentially the same as that described in the RNA Master Blot User Manual, Apendix C (1997, Clontech Laboratories, Palo Alto, CA, USA). In brief, expression levels of the five housekeeping genes in all tissue samples were measured in three independent reactions per gene using the LightCycler and a constant amount (25 .mu.g) of starting RNA. The calculated copy numbers for each gene, derived from comparison with simultaneously reacted standards of known concentrations, were recorded and converted into a percentage of the sum of the copy numbers of the gene in all tissue samples. Then for each tissue sample, the sum of the percentage values for each gene was calculated, and a normalization factor was calculated by dividing the sum percentage value for each tissue by the sum percentage value of one of the tissues arbitrarily selected as a standard. To normalize an experimentally obtained value for the expression of a particular gene in a tissue sample, the obtained value was multiplied by the normalization factor for the tissue tested.

Results are shown in Fig. 11, showing the experimentally obtained copy numbers of mRNA per 10 ng of first-strand cDNA on the left and the normalized values on the right. RNAs used for the cDNA synthesis, along with their supplier and catalog numbers are shown in table 1.

Table 1. Whole-body-screen tissues

Tissue	Supplier	Panel name and catalog number
1. brain	Clontech	Human Total RNA Panel I, K4000-1
2. heart	Clontech	Human Total RNA Panel I, K4000-1
3. kidney	Clontech	Human Total RNA Panel I, K4000-1
4. liver	Clontech	Human Total RNA Panel I, K4000-1
5. lung	Clontech	Human Total RNA Panel I, K4000-1
6. trachea	Clontech	Human Total RNA Panel I, K4000-1
7. bone marrow	Clontech	Human Total RNA Panel II, K4001-1
8. colon	Clontech	Human Total RNA Panel II, K4001-1
9. small intestine	Clontech	Human Total RNA Panel II, K4001-1
10. spleen	Clontech	Human Total RNA Panel II, K4001-1
11. stomach	Clontech	Human Total RNA Panel II, K4001-1
12. thymus	Clontech	Human Total RNA Panel II, K4001-1
13. mammary gland	Clontech	Human Total RNA Panel III, K4002-1
14. skeletal muscle	Clontech	Human Total RNA Panel III, K4002-1
15. prostate	Clontech	Human Total RNA Panel III, K4002-1
16. testis	Clontech	Human Total RNA Panel III, K4002-1
17. uterus	Clontech	Human Total RNA Panel III, K4002-1
18. cerebellum	Clontech	Human Total RNA Panel IV, K4003-1
19. fetal brain	Clontech	Human Total RNA Panel IV, K4003-1
20. fetal liver	Clontech	Human Total RNA Panel IV, K4003-1
21. spinal cord	Clontech	Human Total RNA Panel IV, K4003-1
22. placenta	Clontech	Human Total RNA Panel IV, K4003-1
23. adrenal gland	Clontech	Human Total RNA Panel V, K4004-1
24. pancreas	Clontech	Human Total RNA Panel V, K4004-1
25. salivary gland	Clontech	Human Total RNA Panel V, K4004-1
26. thyroid	Clontech	Human Total RNA Panel V, K4004-1

CysLT2-like GPCR is expressed fairly widely with the notable exceptions of liver, skeletal muscle, and bone marrow, where its expression is nearly undetectable. Due to the fact that CysLT2-like GPCR is expressed in both the lung and immune system, its regulation may impact the course of asthma and related diseaes.

Compared with the expression of the related CysLT1 receptor (shown below), CysLT2-like GPCR is expressed at much lower levels on average in the tissues tested (roughly one-tenth the amount per cell) and shows relatively more pronounced expression in the placenta.

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EXAMPLE 17

Quantitative analysis of relative expression of CysLT2-like GPCR in human tissues

Quantitative expression profiling was performed by the form of quantitative PCR analysis called "kinetic analysis" firstly described in Higuchi et al., 1992 and Higuchi et al., 1993. The principle is that at any given cycle within the exponential phase of PCR, the amount of product is proportional to the initial number of template copies.

If the amplification is performed in the presence of an internally quenched fluorescent oligonucleotide (TaqMan probe) complementary to the target sequence, the probe is cleaved by the 5'-3' endonuclease activity of Taq DNA polymerase and a fluorescent dye released in the medium (Holland et al.). Since the fluorescence emission will increase in direct proportion to the amount of the specific amplified product, the exponential growth phase of PCR product can be detected and used to determine the initial template concentration (Heid et al., 1996, and Gibson et al., 1996).

The amplification of an endogenous control can be performed to standardise the amount of sample RNA added to a reaction. In this kind of experiments the control of choice is the 18S ribosomal RNA. Since reporter dyes with differing emission spectra are available, the target and the endogenous control can be independently quantified in the same tube if probes labelled with different dyes are used.

All "real time PCR" measurements of fluorescence are made in the ABI Prism 7700 Sequence detector System (PE Applied Biosystems, Foster City, CA).

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cDNA preparation

The total RNAs used for expression quantification are listed in Table 2 along with their purchasers.

Fifty □gs of each RNA were treated with DNase I for 1 hour at 37°C in the following reaction mix:

DNase I, RNase-free (Roche Diagnostics, Germany)	0.2 U/μL
Rnase inhibitor (PE Applied Biosystems, CA)	0.4 U/μL
Tris-HCl pH 7.9	10mM
$MgCl_2$	10mM
NaCl	50mM
DTT	1mM

After incubation, RNA was extracted once with 1 volume of phenol:chloroform:isoamyl alcohol (24:24:1) and once with chloroform, and precipitated with 1/10 volume of NaAcetate 3M pH5.2 and 2 volume ethanol.

After spectrophotometric quantification, each sample has been reverse transcribed with the TaqMan Reverse Transcription Reagents (PE Applied Biosystems, CA) accordingly to purchaser protocol. RNA final concentration in the reaction mix was 200ng/μL. Reverse transcription was made with 2.5μM of random hexamers.

TaqMan quantitative analysis

Specific primers and probe were designed accordingly to PE Applied Biosystems recommendations and are listed below:
forward primer: 5'-TTCCTGACCGTGCTGAGTGTT-3'
reverse primer: 5'-GTGACATGCAGAAGCCGAAAG-3'
probe: 5'-(FAM) TGCGTTTCCTGGCAATGGTTCACC (TAMRA)-3'
where FAM = 6-carboxy-fluorescein
and TAMRA = 6-carboxy-tetramethyl-rhodamine.
The expected length of the PCR product was 68bp.

Quantification experiments were performed on 50 ng of reverse transcribed RNA from each sample. Each determination was done in triplicate.

Total cDNA content was normalized with the simultaneous quantification (multiplex PCR) of the 18S ribosomal RNA by use of the Pre-Developed TaqMan Assay Reagents (PDAR) Control Kit (PE Applied Biosystems, CA).

Assay reaction mix was as follows:

	final
TaqMan Universal PCR Master Mix (2x) (PE Applied Biosystems, CA)	1x
PDAR control – 18S RNA (20x	1x
Forward primer	300nM
Reverse primer	900nM
Probe	200nM
cDNA	10ng
Water	to 25µL

PCR conditions were:

1 time the following steps:	
pre PCR	2' at 50° C
	10' at 95°C
40 times the following steps:	
denaturation	15'' at 95°C
annealing/extension	1' at 60°C

The experiment was performed on an ABI Prism 7700 Sequence Detector (PE Applied Biosystems, CA). At the end of the run, fluorescence data acquired during PCR were processed as described in the ABI Prism 7700 user's manual in order to achieve better background subtraction as well as signal linearity with the starting target quantity.

The results obtained are shown in Figures 12, 13 and 14.

TABLE 2

RNA	Purch.&#catalog
h. Fetal Brain	Clontech (CA) 640191
h. Brain	OriGene (MD) HT1001
h. Muscle	OriGene (MD) HT1008
h. Heart	OriGene (MD) HT1002
h. Lung	OriGene (MD) HT1009
h. Kidney	OriGene (MD) HT1003
h. Liver	OriGene (MD) HT1005
h. Thymus	Clontech (CA) 640281
h. Testis	OriGene (MD) HT1011
h. Colon	OriGene (MD) HT1015
h. Placenta	OriGene (MD) HT1013
h. Trachea	Clontech 640911
h. Pancreas	Clontech 640311
h. Gastric Mucose	From autopsy
h. Fetal Liver	Clontech (CA) 640181
h. Bladder	Invitrogen (CA) D602001
h. Prostate	Clontech (CA) 640381
h. Adrenal Gland	Clontech (CA) 640161
h. Spleen	OriGene (MD) HT1004
h. Hypertrophic Prostate	from autopsy
h. Prostate	from autopsy
h. Cerebellum	Clontech (CA) 640351
h. brain	from autopsy
h. Hypothalamus	from autopsy
h. Cortex	from autopsy
h. Amygdala	from autopsy

RNA	Purch.&#catalog
h. Amygdala	from autopsy
h. Cerebellum	from autopsy
h. Hippocampus	from autopsy
h. Choroid plexus	from autopsy
h. Thalamus	from autopsy
h. Spinal Cord	Clontech (CA) K40031
h. DRG	from autopsy

EXAMPLE 18

Effects of CysLT2-like GPCR antagonists on calcium mobilization of cells

Materials and methods

Plasmids, transfection and cell culture

Cloning of cDNAs encoding human CysLT1 receptor (CysLT1R) and human CysLT2 receptor (CysLT2R) were carried out. *HindIII-NotI* fragment (1.2 kb) containing the translation open reading frame (ORF) of CysLT1R and *EcoRI* fragment (1.4 kb) containing the ORF of CysLT2R were subcloned into a mammalian episomal expression plasmid pEAK10 (Edge Biosystems), respectively. The expression plasmids were transfected into PEAK-stable cell (Edge Biosystems) with the use of Lipofectamin Plus (Gibco) according to the manufacturer's instruction. The transfected cells were cultured in D-MEM supplemented with 10% FCS, penicillin/streptomycin/L-glutamin and increasing concentration of puromycin (from 0.5-2 μg/ml) for 3-weeks to select stably transfected cells. The resulting puromycin resistant cells were kept cultured in the medium containing 2 μg/ml puromycin. Mouse preB-cell line L1.2 was cultured in RPMI1640 supplemented with 10% FCS and penicillin/streptomycin/L-glutamin.

Calcium mobilization assay

The transfected cells were loosely attached on culture flask, so suspended by replacing the medium to 293-SFM II (Gibco) and tapping the flask. The cell suspension was washed once with washing solution (Hanks balanced salt solution supplemented with 20 mM Hepes and 0.1% BSA) and loaded with 2 □M Fluo-3 AM (Molecular Probes) in washing solution containing 1 mM probenecid (Sigma) at an ambient temperature for 1 h. After washing once with washing solution containing 1 mM probenecid, the cells were seeded into wells of clear bottomed black 384-well plate (Nunc) at the density of 5,000 cells per well. For the evaluation of antagonists, dilution series of antagonists were added in the wells 5 min before the stimulation. Intracellular calcium mobilization was monitored by FDSS-6000 (Hamamatsu-Photonics). Calcium mobilization data obtained as fluorescence change was calculated as ratio of the initial fluorescence count without stimulation.

Other reagents

Montelukast, pranlukast and Bay y8934 were synthesized. Bay y9773 was purchased from Biomol. Leukotriene D4 (LTD4) and Protease activated receptor-1 (PAR-1) activating peptide (H-Ser-Phe-Lue-Lue-Arg-Asn-NH2) were purchased from Sigma and Bachem, respectively.

The results obtained are shown in Figures 15, 16 and 17.

Figure 15 shows kinetic study of cysLTR-dependent calcium mobilization in the receptor transfected cells. PEAK-stable cells stably transfected with expression plasmids of CysLT2R (A), CysLT1R (B) and vacant vector (C) were stimulated with LTD4 at indicated concentrations. LTD4 was added at 10 sec after the start of measurement. Murine preB cell line L1.2 which expresses endogenous cysLT1R was also tested (D).

LTD-6, LTD-7, LTD-8..... in the figure mean 10^{-6} M, 10^{-7} M, 10^{-8} M..... of LTD_4.

Figure 16 shows effects of CysLTR antagonists on LTD4 induced calcium mobilization in receptor transfected cells. Effects of Bay-y8934, Bay-y9773, montelukast and pranlukact were tested on CysLT2R (upper) or CysLT1R (lower) dependent calcium mobilization. Fifty-second integrals of fluorescence changes by the addition of LTD4 were plotted (Z-axes) against LTD4 concentration (X-axes). Each data is an average of 6 assay points in a single experiment.

Figure 17 shows effects of CysLTR antagonists on LTD4 induced calcium mobilization in receptor transfected cells. The results of Figure 15 were converted to concentration of antagonists (the abscissa) versus % inhibition (the ordinates). The effects of antagonists were evaluated against 2 nM and 0.2 nM LTD4 for CysLT2R and CysLT1R transfected cells, respectively. Protease activated receptor-1 (PAR-1) is an endogenous Gq-coupled receptor in PEAK-stable cells. Ten microM of PAR-1 activating peptide was used to stimulate the receptor on the cells stably transfected with vacant vector. The selected concentration of the agonists gave 50-70% of the maximum response by each receptor.

EXAMPLE 19

Binding and inhibited binding of a specific molecule to CysLT2-like GPCR

Membrane preparation for CysLT2 receptor binding assay

Peak stable cells transformed with CysLT2 expression vector were maintained in D-MEM supplemented with 10% FCS and 2 μg/ml of puromycin. Cells were collected and kept at –80 °C until membrane preparation. Frozen cells were suspended in cold membrane preparation buffer (50 mM Tris-HCl pH 7.5, protease inhibitor mixture(#1873580, Roche)), and disrupted with Polytron (Cat.#PT10-35, Kinematica AG, Switzerland). Disrupted cells were centrifuged at 500 x g for 5 minutes at 4 °C to remove the nuclear fraction, and supernatants were applied to high speed centrifugation (45,000 x g, 15 minutes, 4 °C) to precipitate membrane fraction. After

the supernatant was removed, membrane preparation buffer were added to the pellet and homogenized gently on ice. Glycerol (final conc.;10%) and bovine serum albumin (final conc.:0.5%, Cat#A-3059, Sigma) were added to the membrane suspension as stabilizer. Membrane suspension was then divided into small vials and frozen in liquid nitrogen. After 1 hour freezing, vials were kept at –80 °C until use.

Saturation binding

Leukotriene D_4 (LTD_4) and [^{3}H]-labeled LTD_4 were purchased (Cat.#20310, Cayman Chemical, Cat.#NET-1019, NEN, respectively). For the saturation binding, [^{3}H]-labeled LTD_4 were mixed with non-labeled LTD_4 to reduce the specific radioactivity. For the measurement of the total bindings, 2-fold serially diluted [^{3}H]-labeled LTD_4 and membranes prepared above (final concentration;50 □g/ml) were incubated in 120 □l of binding buffer (50 mM Tris-HCl pH 7.4, 40 mM $MgCl_2$, 5 mM L-Serine, 5 mM Boric acid, 5 mM L-Cysteine, 100 μM S-Hexyl-glutathione, 0.1% BSA) for 2 hours at room temperature using a 96-well polypropylene plate (Cat.#3794, Costar). At the end of the binding reaction, 100 μl of the reaction mixture was transferred to a 96-well filtration plate (Cat.#MAFB-N0B, Millipore), and washed 3 times with 200 μl/well of cold binding buffer. The non-specific binding was determined by parallel incubation in the presence of 2 μM of LTD_4. The specific binding (total binding – non-specific binding) was measured by liquid scintillation counter (TopCount™, Packard). Then, the specific binding was transformed to Scatchard plot to calculate Kd value. In the figure of Scatchard plot, Kd of [^{3}H]LTD_4 to CysLT2 was calculated to be 7.5 nM.

Competition for [^{3}H]LTD_4-specific binding to CysLT2

Leukotriene B_4 (LTB_4), LTC_4, LTD_4 and LTE_4 were purchased (Cat.#20110, Cat.#20210, Cat.#20310, Cat.#20410, Cayman Chemical, respectively). Each of the non-labeled leukotrien, which was 3-fold serially diluted, membranes (final concentration;50 □g/ml), and [^{3}H]-labeled LTD_4 (0.4 nM) were incubated in 120 μl

of binding buffer for 2 hours at room temperature using a 96-well polypropylene plate. At the end of the binding reaction, 100 µl of the reaction mixture was transferred to a 96-well filtration plate (Cat.#MAFB-N0B, Millipore), and washed 3 times with 200 µl/well of cold binding buffer. The non-specific binding was determined by parallel incubation in the presence of 2 µM of LTD_4. The specific binding (total binding – non-specific binding) was measured by liquid scintillation counter (TopCount™, Packard) and expressed as the relative binding (%) in the figure. The IC_{50} values for LTC_4, LTD_4, and LTE_4 were calculated to be 6 nM, 8 nM, and 2000 nM, respectively. However, LTB_4 at concentrations up to 10^{-6} M did not inhibit [^{3}H]LTD_4 binding to CysLT2.

Inhibition assay using several antagonists

Three-fold serially diluted Bay y9773, montelukast, or pranlukast, membranes (final concentration;50 µg/ml), and [^{3}H]-labeled LTD_4 (0.4 nM) were incubated in 120 µl of binding buffer for 2 hours at room temperature using a 96-well polypropylene plate. The specific binding of [^{3}H]LTD_4 were measured by the method described above and expressed as the relative inhibition (%) in the figure. The IC_{50} values for Bay y9773, Bay y8934, montelukast, and pranlukast were calculated to be 2 µM, 1 µM, 30 µM, and 8 µM, respectively.

The results obtained are shown in Figures 18, 19 and 20.

Fig. 18 shows saturation binding of [^{3}H]LTD_4 to the membrane of a CysLT2-expressing stable transfectant (A), and scatchard analysis of the saturation binding shown in A. The Kd of [^{3}H]LTD_4 to CysLT2 is calculated to be 7,5 nM.

Fig. 19 shows competition for [^{3}H]LTD_4 –specific binding to CysLT2. LTC_4, LTD_4 and LTE_4 inhibit [^{3}H]LTD_4 binding to the membrane of a CysLT2-expressing stable transfectant. IC_{50} values for LTC_4, LTD_4 and LTE_4 are 6 nM, 8 nM, and 2000 nM,

respectively. However, LTB_4 at concentrations up to 10^{-6} M did not inhibit [^{3}H]LTD_4 binding.

Fig. 20 shows inhibition assays with several antagonists on [^{3}H]LTD_4/CysLT2 binding. Bay y9773, motelukast, and pranlukast inhibited [^{3}H]LTD_4 binding to the membrane from CysLT2-expressing stable transformant. IC_{50} values for Bay y9773, montelukast, and pranlukast are 2 μM, 30 μM, and 8 μM, respectively.

CLAIMS

1. An isolated polynucleotide encoding a CysLT2-like GPCR polypeptide and being selected from the group consisting of:

a) a polynucleotide encoding a CysLT2-like GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 39% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

b) a polynucleotide comprising the sequence of SEQ ID NOS: 1or 3;

c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);

d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and

e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d).

2. An expression vector containing any polynucleotide of claim 1.

3. A host cell containing the expression vector of claim 2.

4. A substantially purified CysLT2-like GPCR polypeptide encoded by a polynucleotide of claim 1.

5. A method for producing a CysLT2-like GPCR polypeptide, wherein the method comprises the following steps:

a) culturing the host cell of claim 3 under conditions suitable for the expression of the CysLT2-like GPCR polypeptide; and

b) recovering the CysLT2-like GPCR polypeptide from the host cell culture.

6. A method for detection of a polynucleotide encoding a CysLT2-like GPCR polypetide in a biological sample comprising the following steps:

a) hybridizing any polynucleotide of claim 1 to a nucleic acid material of a biological sample, thereby forming a hybridization complex; and

b) detecting said hybridization complex.

7. The method of claim 6, wherein before hybridization, the nucleic acid material of the biological sample is amplified.

8. A method for the detection of a polynucleotide of claim 1 or a CysLT2-like GPCR polypeptide of claim 4 comprising the steps of contacting a biological sample with a reagent which specifically interacts with the polynucleotide or the CysLT2-like GPCR polypeptide.

9. A diagnostic kit for conducting the method of any one of claims 6 to 8.

10. A method of screening for agents which decrease the activity of a CysLT2-like GPCR, comprising the steps of:

contacting a test compound with any CysLT2-like GPCR polypeptide encoded by any polynucleotide of claim 1;

detecting binding of the test compound to the CysLT2-like GPCR polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential therapeutic agent for decreasing the activity of a CysLT2-like GPCR.

11. A method of screening for agents which regulate the activity of a CysLT2-like GPCR, comprising the steps of:

contacting a test compound with a CysLT2-like GPCR polypeptide encoded by any polynucleotide of claim 1; and

detecting a CysLT2-like GPCR activity of the polypeptide, wherein a test compound which increases the CysLT2-like GPCR activity is identified as a potential therapeutic agent for increasing the activity of the CysLT2-like GPCR, and wherein a test compound which decreases the CysLT2-like GPCR activity of the polypeptide is identified as a potential therapeutic agent for decreasing the activity of the CysLT2-like GPCR.

12. A method of screening for agents which decrease the activity of a CysLT2-like GPCR, comprising the steps of:

contacting a test compound with any polynucleotide of claim 1 and

detecting binding of the test compound to the polynucleotide, wherein a test compound which binds to the polynucleotide is identified as a potential therapeutic agent for decreasing the activity of CysLT2-like GPCR.

13. A method of reducing the activity of CysLT2-like GPCR, comprising the steps of:

contacting a cell with a reagent which specifically binds to any polynucleotide of claim 1 or any CysLT2-like GPCR polypeptide of claim 4, whereby the activity of CysLT2-like GPCR is reduced.

14. A reagent that modulates the activity of a CysLT2-like GPCR polypeptide or a polynucleotide wherein said reagent is identified by the method of any of the claims 10 to 12.

15. A pharmaceutical composition, comprising:

the expression vector of claim 2 or the reagent of claim 14 and a pharmaceutically acceptable carrier.

16. Use of the pharmaceutical composition of claim 15 for modulating the activity of a CysLT2-like GPCR in a disease.

17. Use of claim 16 wherein the disease is central or peripheral nervous system disease, asthma or cardiovascular disease.

18. A cDNA encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2.

19. The cDNA of claim 18 which comprises SEQ ID NO:1.

20. The cDNA of claim 18 which consists of SEQ ID NO:1.

21. An expression vector comprising a polynucleotide which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2.

22. The expression vector of claim 21 wherein the polynucleotide consists of SEQ ID NO:1.

23. A host cell comprising an expression vector which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2.

24. The host cell of claim 23 wherein the polynucleotide consists of SEQ ID NO:1.

25. A purified polypeptide comprising the amino acid sequence shown in SEQ ID NO:2.

26. The purified polypeptide of claim 25 which consists of the amino acid sequence shown in SEQ ID NO:2.

27. A fusion protein comprising a polypeptide having the amino acid sequence shown in SEQ ID NO:2.

28. A method of producing a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising the steps of:

culturing a host cell comprising an expression vector which encodes the polypeptide under conditions whereby the polypeptide is expressed; and isolating the polypeptide.

29. The method of claim 28 wherein the expression vector comprises SEQ ID NO:1.

30. A method of detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising the steps of:

hybridizing a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO:1 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and

detecting the hybridization complex.

31. The method of claim 30 further comprising the step of amplifying the nucleic acid material before the step of hybridizing.

32. A kit for detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising:

a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO:1; and instructions for the method of claim 30.

33. A method of detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising the steps of:

contacting a biological sample with a reagent that specifically binds to the polypeptide to form a reagent-polypeptide complex; and

detecting the reagent-polypeptide complex.

34. The method of claim 33 wherein the reagent is an antibody.

35. A kit for detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising:

an antibody which specifically binds to the polypeptide; and

instructions for the method of claim 33.

36. A method of screening for agents which can regulate the activity of a CysLT2-like GPCR protein, comprising the steps of:

contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 39% identical to the amino acid sequence shown in SEQ ID NO:2 and (2) the amino acid sequence shown in SEQ ID NO:2; and

detecting binding of the test compound to the polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential agent for regulating activity of the CysLT2-like GPCR protein.

37. The method of claim 36 wherein the step of contacting is in a cell.

38. The method of claim 36 wherein the cell is *in vitro*.

39. The method of claim 36 wherein the step of contacting is in a cell-free system.

40. The method of claim 36 wherein the polypeptide comprises a detectable label.

41. The method of claim 36 wherein the test compound comprises a detectable label.

42. The method of claim 36 wherein the test compound displaces a labeled ligand which is bound to the polypeptide.

43. The method of claim 36 wherein the polypeptide is bound to a solid support.

44. The method of claim 36 wherein the test compound is bound to a solid support.

45. A method of screening for agents which regulate an activity of a human human CysLT2-like GPCR protein, comprising the steps of:

contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 39% identical to the amino acid sequence shown in SEQ ID NO:2 and (2) the amino acid sequence shown in SEQ ID NO:2; and

detecting an activity of the polypeptide, wherein a test compound which increases the activity of the polypeptide is identified as a potential agent for increasing the activity of the human CysLT2-like GPCR protein, and wherein a test compound which decreases the activity of the polypeptide is identified as a potential agent for decreasing the activity of the human CysLT2-like GPCR protein.

46. The method of claim 45 wherein the step of contacting is in a cell.

47. The method of claim 45 wherein the cell is *in vitro*.

48. The method of claim 45 wherein the step of contacting is in a cell-free system.

49. The method of claim 45 wherein the activity is cyclic AMP formation.

50. The method of claim 45 wherein the activity is mobilization of intracellular calcium.

51. The method of claim 45 wherein the activity is phosphoinositide metabolism.

52. A method of screening for agents which regulate an activity of a human CysLT2-like GPCR protein, comprising the steps of:

contacting a test compound with a product encoded by a polynucleotide which comprises the nucleotide sequence shown in SEQ ID NO:1; and

detecting binding of the test compound to the product, wherein a test compound which binds to the product is identified as a potential agent for regulating the activity of the human CysLT2-like GPCR protein.

53. The method of claim 52 wherein the product is a polypeptide.

54. The method of claim 52 wherein the product is RNA.

55. A method of reducing activity of a human CysLT2-like GPCR protein, comprising the step of:

contacting a cell with a reagent which specifically binds to a product encoded by a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO:1, whereby the activity of a human CysLT2-like GPCR protein is reduced.

56. The method of claim 55 wherein the product is a polypeptide.

57. The method of claim 56 wherein the reagent is an antibody.

58. The method of claim 55 wherein the product is RNA.

59. The method of claim 58 wherein the reagent is an antisense oligonucleotide.

60. The method of claim 58 wherein the reagent is a ribozyme.

61. The method of claim 55 wherein the cell is *in vitro*.

62. The method of claim 55 wherein the cell is *in vivo*.

63. A pharmaceutical composition, comprising:

a reagent which specifically binds to a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2; and

a pharmaceutically acceptable carrier.

64. The pharmaceutical composition of claim 63 wherein the reagent is an antibody.

65. A pharmaceutical composition, comprising:

a reagent which specifically binds to a product of a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO:1; and

a pharmaceutically acceptable carrier.

66. The pharmaceutical composition of claim 65 wherein the reagent is a ribozyme.

67. The pharmaceutical composition of claim 65 wherein the reagent is an antisense oligonucleotide.

68. The pharmaceutical composition of claim 65 wherein the reagent is an antibody.

69. A pharmaceutical composition, comprising:

an expression vector encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2; and

a pharmaceutically acceptable carrier.

70. The pharmaceutical composition of claim 69 wherein the expression vector comprises SEQ ID NO:1.

71. A method of treating CysLT2-like GPCR, comprising the step of: ,

administering to a patient in need thereof a therapeutically effective dose of a reagent that inhibits a function of a human CysLT2-like GPCR protein, whereby symptoms of the CysLT2-like GPCR are ameliorated.

72. The method of claim 71 wherein the reagent is identified by the method of claim 36.

73. The method of claim 71 wherein the reagent is identified by the method of claim 45.

74. The method of claim 71 wherein the reagent is identified by the method of claim 52.

75. A method of treating a CysLT2-like GPCR disorder, comprising the step of:

administering to a patient in need thereof a therapeutically effective dose of a reagent that inhibits a function of a human CysLT2-like GPCR protein, whereby symptoms of the CysLT2-like GPCR disorder are ameliorated.

76. The method of claim 75 wherein the reagent is identified by the method of claim 36.

77. The method of claim 75 wherein the reagent is identified by the method of claim 45.

78. The method of claim 75 wherein the reagent is identified by the method of claim 52.

79. The method of claim 75 wherein the CysLT2-like GPCR disorder is peripheral or central nervous system disease, asthma or cardiovascular disease.

FIG. 1

BLASTP - Query = 38_TR1; Hit = swiss|Q13304|GPRH_HUMAN

This hit is scoring at : 2e-45 (expectation value)
Alignment length (overlap) : 317
Identities : 35 %
Scoring matrix : BLOSUM62 (used to infer consensus pattern)
Database searched : nrdb

```
Q:     16 EMEPNG---TFSNNNSRNCTIEN-FKREFFPIVYLIIFFWGVLGNGLSIYVFLQPYKKST
          E:.P G   .FS  .:..C  E. .:. .F. .YL: F. .::GN L::::F::.:K..T
H:      5 EVAPPGLITNFSLATAEQCGQETPLENMLFASFYLLDFILALVGNTLALWLFIRDHKSGT

          SVNVFMLNLAISDLLFISTLPFRADYYLRGSNWIFGDLACRIMSYSLYVNMYSSIYFLTV
          ..NVF:::LA::DL  : .LP R. Y:..G::W FG::ACR:..: .Y:NMY:SIYFLT.
          PANVFLMHLAVADLSCVLVLPTRLVYHFSGNHWPFGEIACRLTGFLFYLNMYASIYFLTC

          LSVVRFLAMVHPFRLLHVTSIRSAWILCGIIWILI-MASSIMLLDSGSEQNGSVTSCLEL
          :S. RFLA:VHP.: L.:..   A :.C..:W::: :A.: :L:.. : Q..... CL:
          ISADRFLAIVHPVKSLKLRRPLYAHLACAFLWVVVAVAMAPLLVSPQTVQTNHTVVCLQ-

          NLYKIAKLQTMNYIALVVGCLLPFFTLSICYLLIIRVLLKVEVPESGLRVSHR---KALT
           LY: .K..   .::L.V. ..PF.T. .CYLLIIR L      ..GLRV..R   KA:.
          -LYR-EKASHHALVSLAVAFTFPFITTVTCYLLIIRSL------RQGLRVEKRLKTKAVR

          TIIITLIIFFLCFLPYHTLRTVHLTTWKV--GLCKDRLHKALV--ITLALAAANACFNPL
          .I.I.L.IF.:CF:PYH. R:V::. ::   . C..:   AL.  IT .L.:.N...:P:
          MIAIVLAIFLVCFVPYHVNRSVYVLHYRSHGASCATQRILALANRITSCLTSLNGALDPI

          LYYFAGENFKDRLKSAL     320
          :Y:F..E.F:..L :.L
          MYFFVAEKFRHALCNLL     312
```

Transmembrane Helix
Prosite GPCR signature
BLOCKS GPCR regions

Transmembranehelix detected in 38_TR1 by TM-Helix:
41 to 63, 77 to 96, 113 to 137, 156 to 173, 202 to 223, 249 to 268, 291 to 308

FIG. 2

BLOCKS serach results:

AC#	Description	Strength	Score
BL00237A	0 G-protein coupled receptors proteins.	1258	1266
AA#	103 WIFGDLACRIMSYSLYVNMYSSIYFLTVLSVVRFLAMVHP		
BL00237D	0 G-protein coupled receptors proteins.	1222	1231
AA#	296 NACFNPLLYYFAGENFK		
BL00237C	0 G-protein coupled receptors proteins.	1172	1138
AA#	239 VSHRKALTTIIITLIIFFLCFLPYHTL		
BL00237B	0 G-protein coupled receptors proteins.	1100	1045
AA#	209 CLLPFFTLSICY		

FIG. 3

BLASTP - Query = 38_TR1; Hit = trembl|AF119711|AF119711_1

gene: "CYSLT1"; product: "cysLT1 LTD4 receptor"; Homo sapiens cysLT1 LTD4 receptor (CYSLT1) mRNA, complete cds.

This hit is scoring at : 2e-55 (expectation value)
Alignment length (overlap) : 298
Identities : 38 %
Scoring matrix : BLOSUM62 (used to infer consensus pattern)
Database searched : nrdb

```
Q:     32 TIENFKREFFPIVYLIIFFWGVLGNGLSIYVFLQPYKKSTSVNVFMLNLAISDLLFISTL
          TI::F:.:.:..:Y :I . G..GNG. :YV.:::.Y.K.::..V:M:NLA::DLL :.TL
H:     17 TIDDFRNQVYSTLYSMISVVGFFGNGFVLYVLIKTYHKKSAFQVYMINLAVADLLCVCTL

          PFRADYYLRGSNWIFGDLACRIMSYSLYVNMYSSIYFLTVLSVVRFLAMVHPFRLLHVTS
          P.R. YY:. . W:FGD..CR:.:Y:LYVN:Y.SI:F:T.:S..R :A:V.P.: :::.:
          PLRVVYYVHKGIWLFGDFLCRLSTYALYVNLYCSIFFMTAMSFFRCIAIVFPVQNINLVT

          IRSAWILCGIIWI-LIMASSIMLLDSGSEQNGSVTSCLELNLYKIAK--LQTMNYIALVV
           :.A .:C  IWI :I:.SS .L: . .:.. : T.C.E    . .K  : .::Y::L.V
          QKKARFVCVGIWIFVILTSSPFLMAKPQKDEKNNTKCFEPPQDNQTKNHVLVLHYVSLFV

          GCLLPFFTLSICYLLIIRVLLKVEVPESGLRVSHRKALTTIIITLIIFFLCFLPYHTLRT
          G ::PF..: :CY.:II .LLK .:.::    SH:KA: .I::....F.:.F:PYH. RT
          GFIIPFVIIIVCYTMIILTLLKKSMKKN--LSSHKKAIGMIMVVTAAFLVSFMPYHIQRT

          VHLTTW--KVGLCKD--RLHKALVITLALAAANACFNPLLYYFAGENFKDRLKSALRK     322
          :HL     :.  C..  R:.K::VITL:LAA:N.CF:PLLY:F:G NF:.RL S..RK
          IHLHFLHNETKPCDSVLRMQKSVVITLSLAASNCCFDPLLYFFSGGNFRKRL-STFRK     311
```

FIG. 4

BLASTP - Query = 38_TR1; Hit = trembl|Y12546|HSP2YLG_1

product: "CysLT2-likeG-protein coupled receptor"; H.sapiens mRNA for CysLT2-likeG-protein coupled receptor

This hit is scoring at : 1e-45 (expectation value)
Alignment length (overlap) : 322
Identities : 34 %
Scoring matrix : BLOSUM62 (used to infer consensus pattern)
Database searched : nrdb

```
Q:     11 SISVSEMEPNG---TFSNNNSRNCTIEN-FKREFFPIVYLIIFFWGVLGNGLSIYVFLQP
          S::  E:.P G   .FS  .:..C  E. .:. .F. .YL: F. .::GN L::::F::.
H:     28 SMNGLEVAPPGLITNFSLATAEQCGQETPLENMLFASFYLLDFILALVGNTLALWLFIRD

          YKKSTSVNVFMLNLAISDLLFISTLPFRADYYLRGSNWIFGDLACRIMSYSLYVNMYSSI
          :K..T..NVF:::LA::DL  : .LP R. Y:..G::W FG::ACR:..: .Y:NMY:SI
          HKSGTPANVFLMHLAVADLSCVLVLPTRLVYHFSGNHWPFGEIACRLTGFLFYLNMYASI

          YFLTVLSVVRFLAMVHPFRLLHVTSIRSAWILCGIIWILI-MASSIMLLDSGSEQNGSVT
          YFLT.:S. RFLA:VHP.: L.:..   A :.C..:W::: :A.: :L:.. : Q.....
          YFLTCISADRFLAIVHPVKSLKLRRPLYAHLACAFLWVVVAVAMAPLLVSPQTVQTNHTV

          SCLELNLYKIAKLQTMNYIALVVGCLLPFFTLSICYLLIIRVLLKVEVPESGLRVSHR--
           CL:  LY: .K..   .::L.V. ..PF.T. .CYLLIIR L      ..GLRV..R
          VCLQ--LYR-EKASHHALVSLAVAFTFPFITTVTCYLLIIRSL------RQGLRVEKRLK

          -KALTTIIITLIIFFLCFLPYHTLRTVHLTTWKV--GLCKDRLHKALV--ITLALAAANA
           KA:..I.I.L.IF.:CF:PYH. R:V::. ::   . C..:   AL.  IT .L.:.N.
          TKAVRMIAIVLAIFLVCFVPYHVNRSVYVLHYRSHGASCATQRILALANRITSCLTSLNG

          CFNPLLYYFAGENFKDRLKSAL     320
          ..:P::Y:F..E.F:..L :.L
          ALDPIMYFFVAEKFRHALCNLL     340
```

FIG. 5.

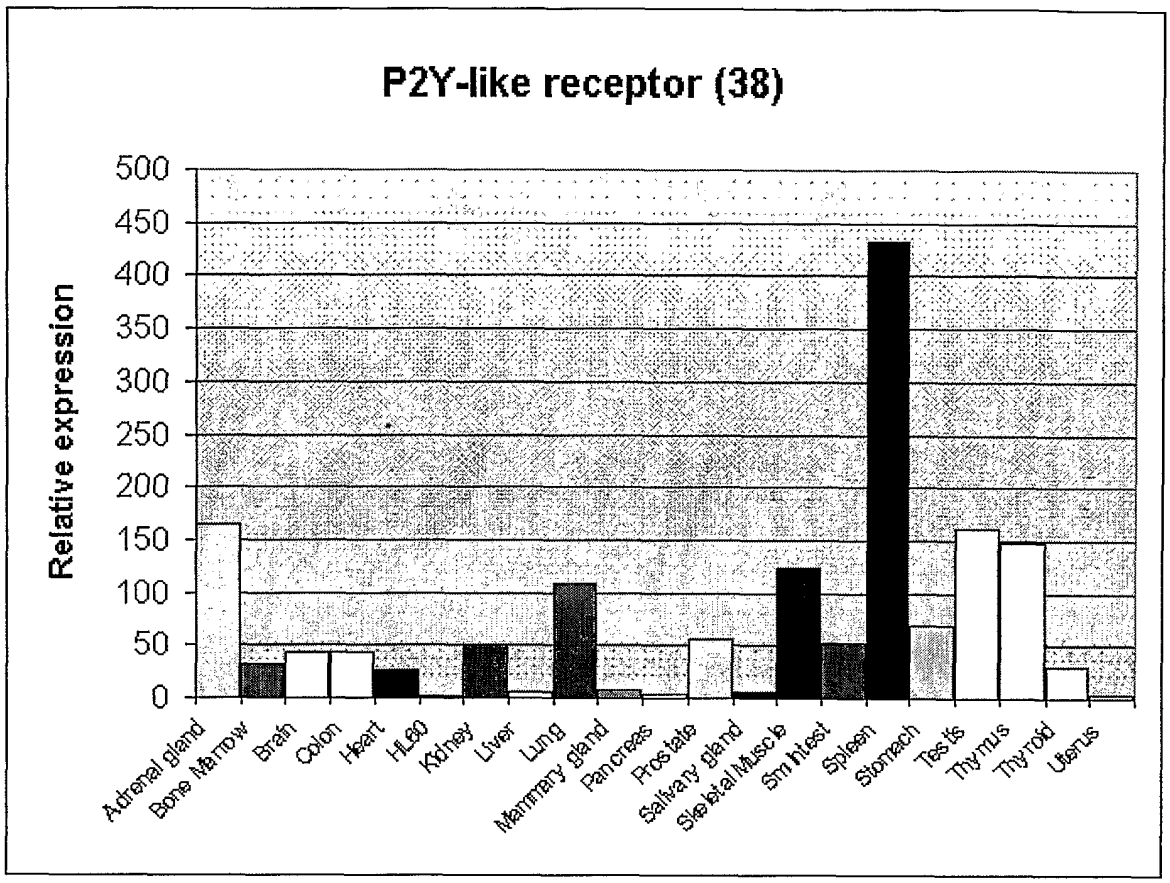
P2Y-like receptor (38)
Relative expression
500
450
400
350
300
250
200
150
100
50
0
Adrenal gland
Bone Marrow
Brain
Colon
Heart
HL60
Kidney
Liver
Lung
Mammary gland
Pancreas
Prostate
Salivary gland
Skeletal Muscle
Sm Intest
Spleen
Stomach
Testis
Thymus
Thyroid
Uterus

FIG. 6.

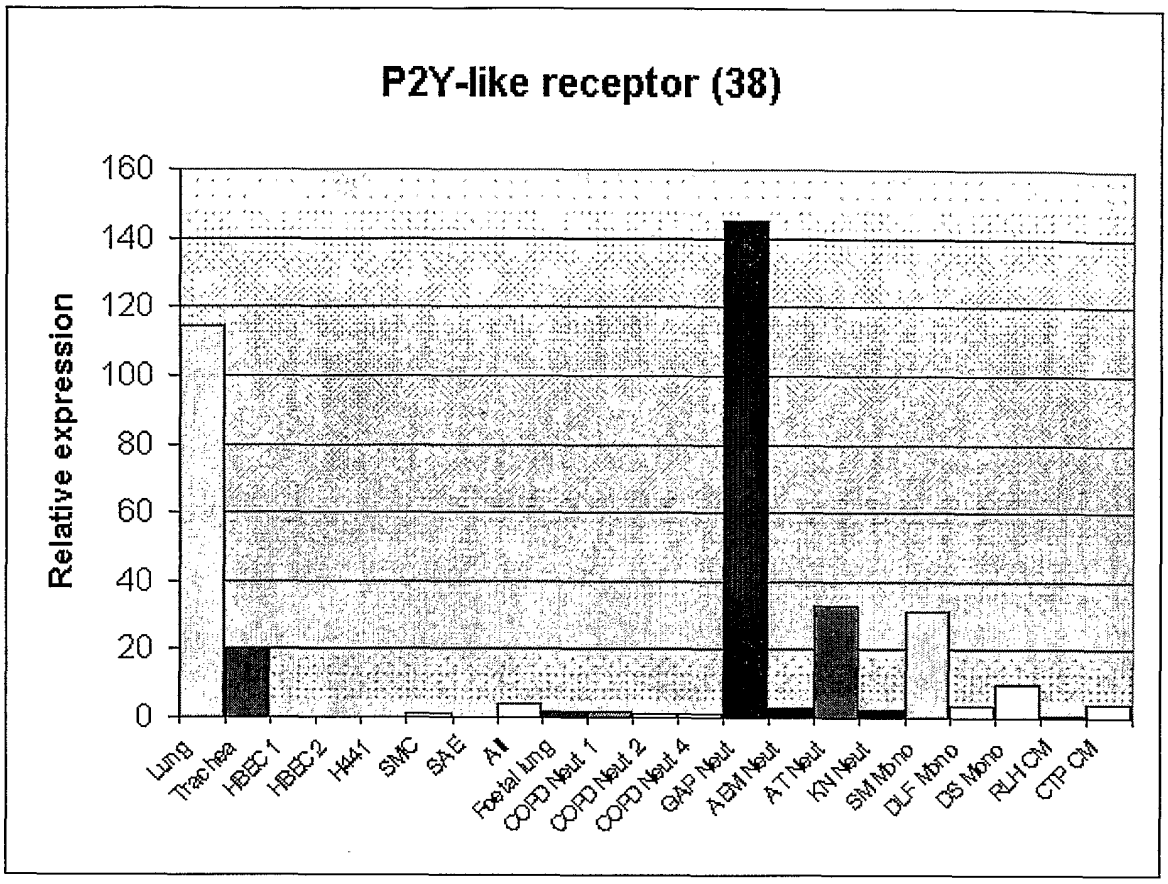
P2Y-like receptor (38)
Relative expression
160
140
120
100
80
60
40
20
0
Lung
Trachea
HBEC 1
HBEC 2
H441
SMC
SAE
AI
Foetal Lung
COPD Neut 1
COPD Neut 2
COPD Neut 4
GAP Neut
AEM Neut
AT Neut
KN Neut
SM Mono
DLF Mono
DS Mono
RLH CM
CTP CM

FIG. 7

MERKFMSLQP SISVSEMEPN GTFSNNNSRN CTIENFKREF **FPIVYLIIFF WGVLGNGLSI**
YVFLQPYKKS TSVNV**FMLNL AISDLLFIST LPFRAD**YYLR GSNWIFGDLA CR**IMSYSLYV**
NMYSSIYFLT VLSVVRFLAM VHPFRLLHVT SIRSA**WILCG IIWILIMASS IML**LDSGSEQ
NGSVTSCLEL NLYKIAKLQT M**NYIALVVGC LLPFFTLSIC YLL**IIRVLLK VEVPESGLRV
SHRKALTTII **ITLIIFFLCF LPYHTLR**TVH LTTWKVGLCK DRLHKALVIT **LALAAANACF**
NPLLYYFAGE NFKDRLKSAL RKGHPQKAKT KCVFPVSVWL RKETRV

Transmembrane helix

BLOCKS GPCR regions

Fig. 8

ATGGAGAGAAAATTTATGTCCTTGCAACCATCCATCTCCGTATCAGAA

ATGGAACCAAATGGCACCTTCAGCAATAACAACAGCAGGAACTGCACAAT

TGAAAACTTCAAGAGAGAATTTTTCCCAATTGTATATCTGATAATATTTT

TCTGGGGAGTCTTGGGAAATGGGTTGTCCATATATGTTTTCCTGCAGCCT

TATAAGAAGTCCACATCTGTGAACGTTTTCATGCTAAATCTGGCCATTTC

AGATCTCCTGTTCATAAGCACGCTTCCCTTCAGGGCTGACTATTATCTTA

GAGGCTCCAATTGGATATTTGGAGACCTGGCCTGCAGGATTATGTCTTAT

TCCTTGTATGTCAACATGTACAGCAGTATTTATTTCCTGACCGTGCTGAG

TGTTGTGCGTTTCCTGGCAATGGTTCACCCCTTTCGGCTTCTGCATGTCA

CCAGCATCAGGAGTGCCTGGATCCTCTGTGGGATCATATGGATCCTTATC

ATGGCTTCCTCAATAATGCTCCTGGACAGTGGCTCTGAGCAGAACGGCAG

TGTCACATCATGCTTAGAGCTGAATCTCTATAAAATTGCTAAGCTGCAGA

CCATGAACTATATTGCCTTGGTGGTGGGCTGCCTGCTGCCATTTTTCACA

CTCAGCATCTGTTATCTGCTGATCATTCGGGTTCTGTTAAAAGTGGAGGT

CCCAGAATCGGGGCTGCGGGTTTCTCACAGGAAGGCACTGACCACCATCA

TCATCACCTTGATCATCTTCTTCTTGTGTTTCCTGCCCTATCACACACTG

AGGACCGTCCACTTGACGACATGGAAAGTGGGTTTATGCAAAGACAGACT

GCATAAAGCTTTGGTTATCACACTGGCCTTGGCAGCAGCCAATGCCTGCT

TCAATCCTCTGCTCTATTACTTTGCTGGGGAGAATTTTAAGGACAGACTA

AAGTCTGCACTCAGAAAAGGCCATCCACAGAAGGCAAAGACAAAGTGTGT

TTTCCCTGTTAGTGTGTGGTTGAGAAAGGAAACAAGAGTATAA

Fig. 9

MERKFMSLQP SISVSEMEPN GTFSNNNSRN CTIENFKREF FPIVYLIIFF WGVLGNGLSI

YVFLQPYKKS TSVNVFMLNL AISDLLFIST LPFRADYYLR GSNWIFGDLA CRIMSYSLYV

NMYSSIYFLT VLSVVRFLAM VHPFRLLHVT SIRSAWILCG IIWILIMASS IMLLDSGSEQ

NGSVTSCLEL NLYKIAKLQT MNYIALVVGC LLPFFTLSIC YLLIIRVLLK VEVPESGLRV

SHRKALTTII ITLIIFFLCF LPYHTLRTVH LTTWKVGLCK DRLHKALVIT LALAAANACF

NPLLYYFAGE NFKDRLKSAL RKGHPQKAKT KCVFPVSVWL RKETRV

Fig. 10

GTTTGAAGCGTCAGCTTCAACCAAACAAATTAATGGCTATTCTACATTCA

AAAATCAGGAAATTTAAATTTATTATGAAATGTAATGCAGCATGTAGTAA

AGACTTAACCAGTGTTTTAAAACTCAACTTTCAAAGAAAAGATAGTATTG

CTCCCTGTTTCATTAAAACCTAGAGAGATGTAATCAGTAAGCAAGAAGGA

AAAAGGGAAATTCACAAAGTAACTTTTTGTGTCTGTTTCTTTTTAACCCA

GCATGGAGAGAAAATTTATGTCCTTGCAACCATCCATCTCCGTATCAGAA

ATGGAACCAAATGGCACCTTCAGCAATAACAACAGCAGGAACTGCACAAT

TGAAAACTTCAAGAGAGAATTTTTCCCAATTGTATATCTGATAATATTTT

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AGATCTCCTGTTCATAAGCACGCTTCCCTTCAGGGCTGACTATTATCTTA

GAGGCTCCAATTGGATATTTGGAGACCTGGCCTGCAGGATTATGTCTTAT

TCCTTGTATGTCAACATGTACAGCAGTATTTATTTCCTGACCGTGCTGAG

TGTTGTGCGTTTCCTGGCAATGGTTCACCCCTTTCGGCTTCTGCATGTCA

CCAGCATCAGGAGTGCCTGGATCCTCTGTGGGATCATATGGATCCTTATC

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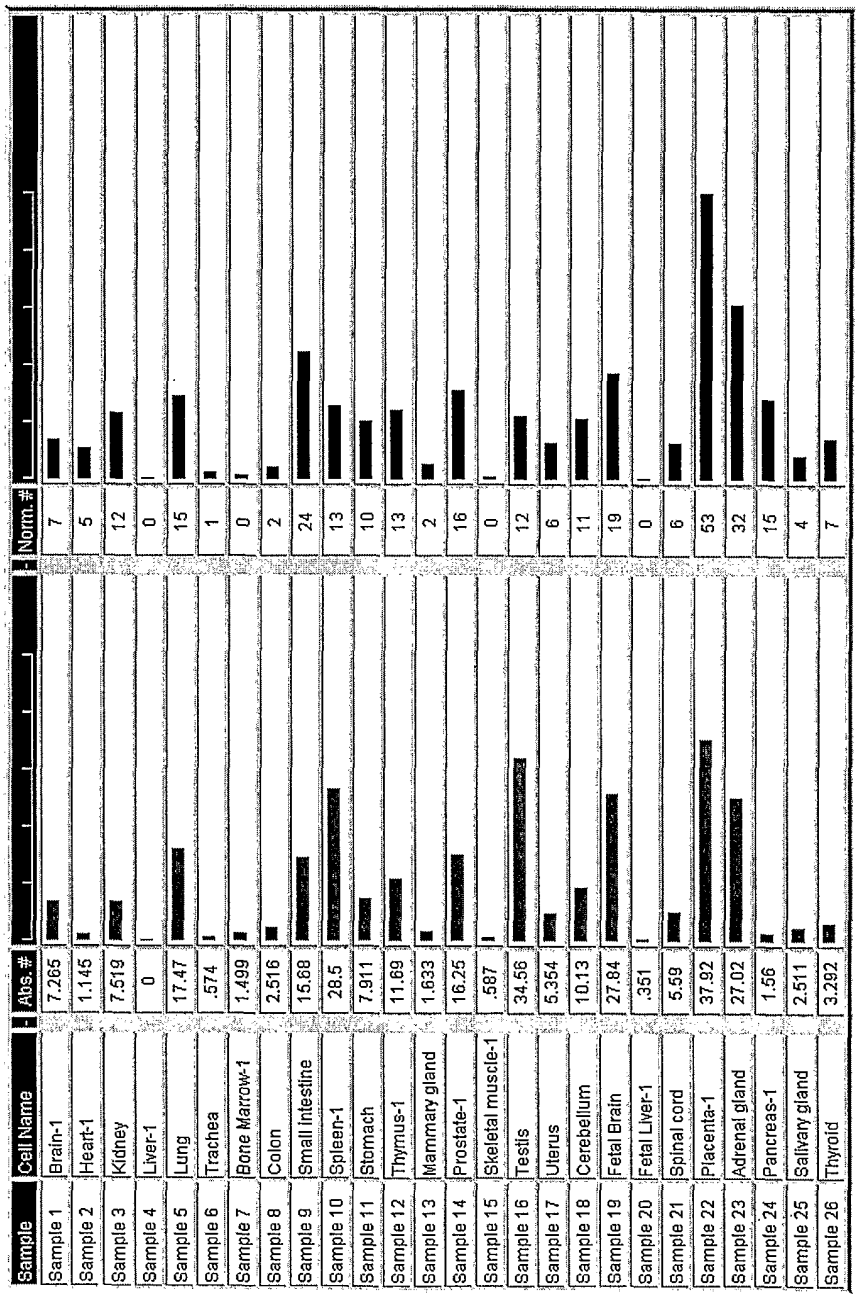
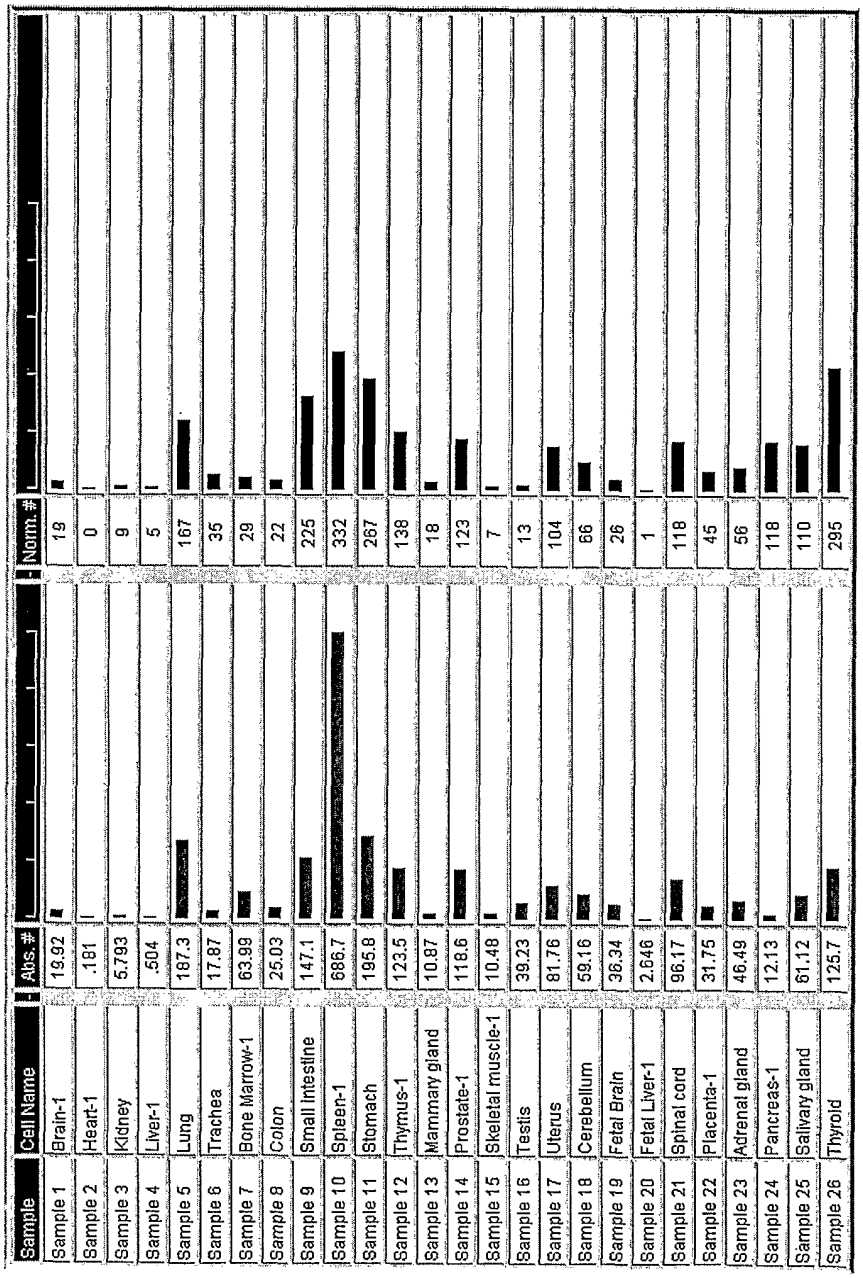


Sample	Cell Name	Abs. #	Norm. #
Sample 1	Brain-1	7.265	7
Sample 2	Heart-1	1.145	5
Sample 3	Kidney	7.519	12
Sample 4	Liver-1	0	0
Sample 5	Lung	17.47	15
Sample 6	Trachea	.574	1
Sample 7	Bone Marrow-1	1.499	0
Sample 8	Colon	2.516	2
Sample 9	Small Intestine	15.68	24
Sample 10	Spleen-1	28.5	13
Sample 11	Stomach	7.911	10
Sample 12	Thymus-1	11.69	13
Sample 13	Mammary gland	1.633	2
Sample 14	Prostate-1	16.25	16
Sample 15	Skeletal muscle-1	.587	0
Sample 16	Testis	34.56	12
Sample 17	Uterus	5.354	6
Sample 18	Cerebellum	10.13	11
Sample 19	Fetal Brain	27.84	19
Sample 20	Fetal Liver-1	.351	0
Sample 21	Spinal cord	5.59	6
Sample 22	Placenta-1	37.92	53
Sample 23	Adrenal gland	27.02	32
Sample 24	Pancreas-1	1.56	15
Sample 25	Salivary gland	2.511	4
Sample 26	Thyroid	3.292	7

Fig. 11 A

FIG. 11 B



Sample	Cell Name	Abs. #	Norm. #
Sample 1	Brain-1	19.92	19
Sample 2	Heart-1	.181	0
Sample 3	Kidney	5.793	9
Sample 4	Liver-1	.504	5
Sample 5	Lung	187.3	167
Sample 6	Trachea	17.87	35
Sample 7	Bone Marrow-1	63.99	29
Sample 8	Colon	25.03	22
Sample 9	Small Intestine	147.1	225
Sample 10	Spleen-1	686.7	332
Sample 11	Stomach	195.8	267
Sample 12	Thymus-1	123.5	138
Sample 13	Mammary gland	10.87	18
Sample 14	Prostate-1	118.6	123
Sample 15	Skeletal muscle-1	10.48	7
Sample 16	Testis	39.23	13
Sample 17	Uterus	81.76	104
Sample 18	Cerebellum	59.16	66
Sample 19	Fetal Brain	36.34	26
Sample 20	Fetal Liver-1	2.646	1
Sample 21	Spinal cord	96.17	118
Sample 22	Placenta-1	31.75	45
Sample 23	Adrenal gland	46.49	56
Sample 24	Pancreas-1	12.13	118
Sample 25	Salivary gland	61.12	110
Sample 26	Thyroid	125.7	295

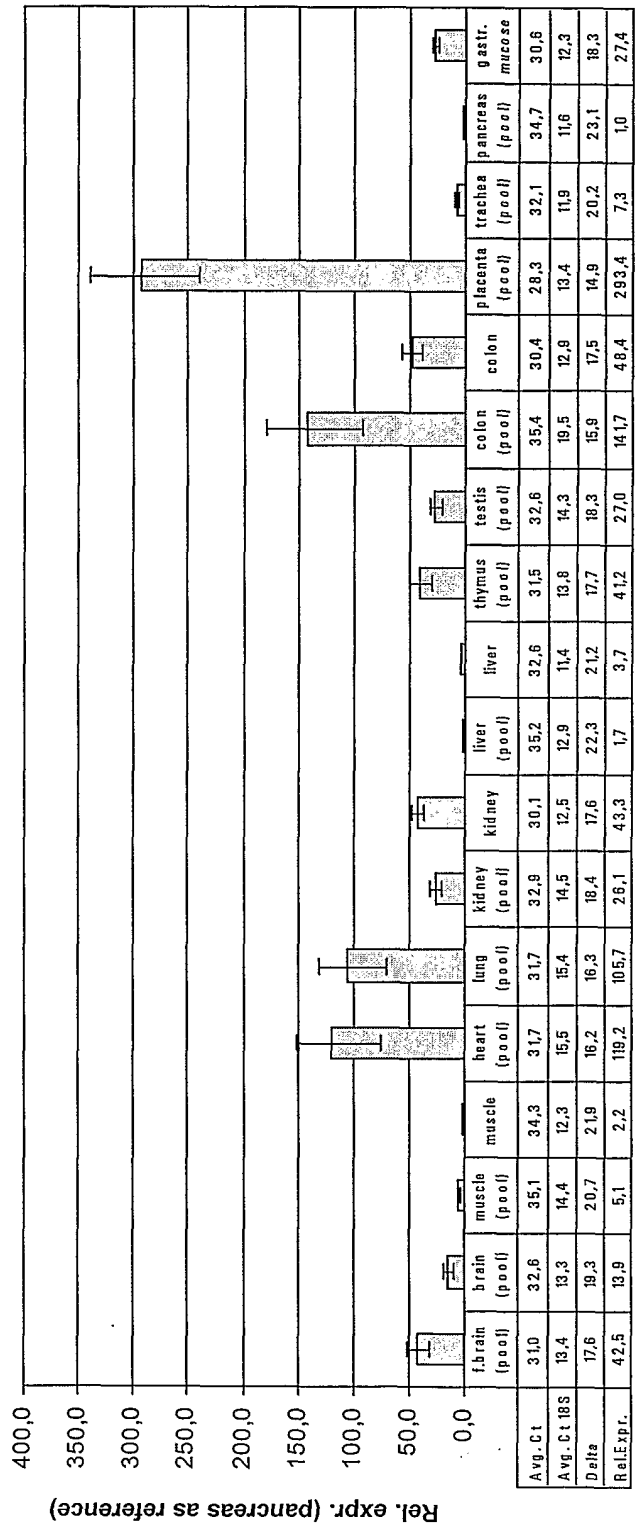
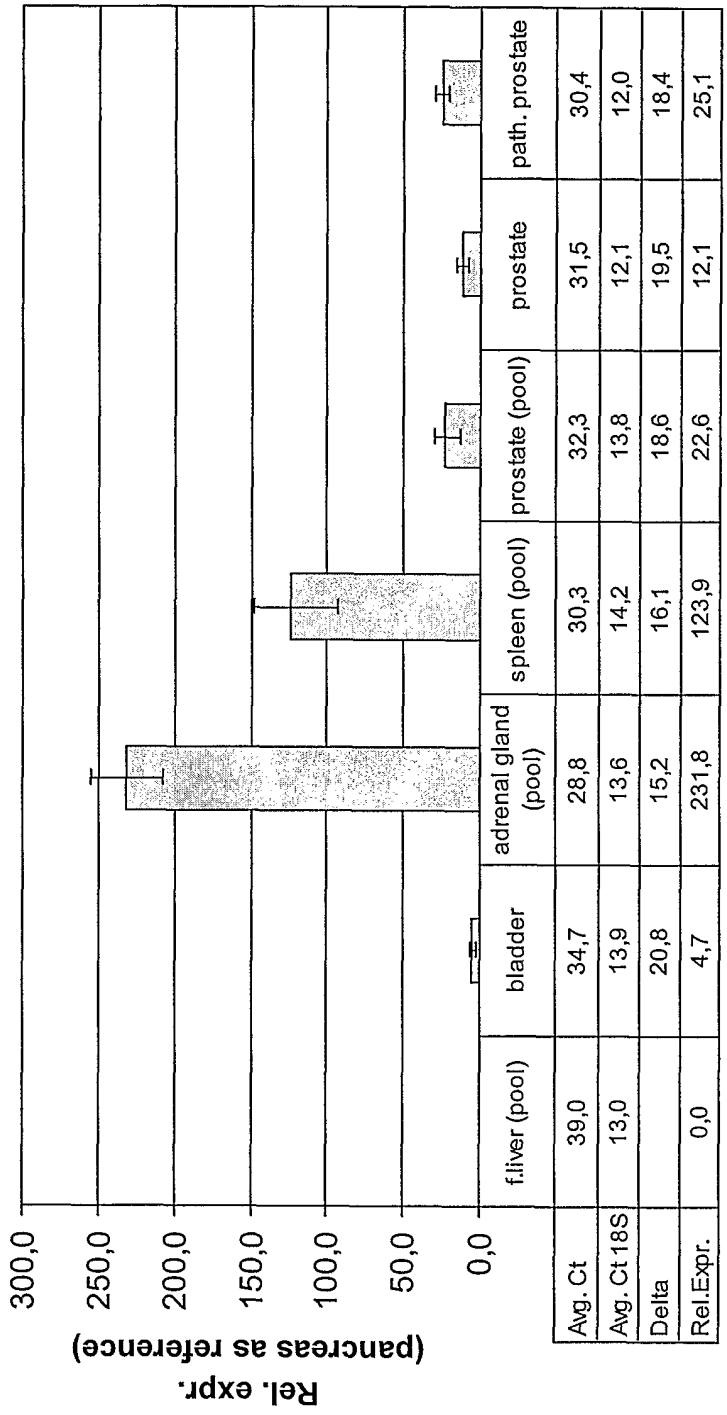

CysLT2-like GPCR (endog. ref. 18S)
Rel. expr. (pancreas as reference)
400,0
350,0
300,0
250,0
200,0
150,0
100,0
50,0
0,0
f.brain (pool)
brain (pool)
muscle (pool)
muscle
heart (pool)
lung (pool)
kidney (pool)
kidney
liver (pool)
liver
thymus (pool)
testis (pool)
colon (pool)
colon
placenta (pool)
trachea (pool)
pancreas (pool)
gastr. mucose
Avg. Ct 31,0 32,6 35,1 34,3 31,7 31,7 32,9 30,1 35,2 32,6 31,5 32,6 35,4 30,4 28,3 32,1 34,7 30,6
Avg. Ct 18S 13,4 13,3 14,4 12,3 15,5 15,4 14,5 12,5 12,9 11,4 13,8 14,3 19,5 12,9 13,4 11,9 11,6 12,3
Delta 17,6 19,3 20,7 21,9 16,2 16,3 18,4 17,6 22,3 21,2 17,7 18,3 15,9 17,5 14,9 20,2 23,1 18,3
Rel.Expr. 42,5 13,9 5,1 2,2 119,2 105,7 26,1 43,3 1,7 3,7 41,2 27,0 141,7 48,4 293,4 7,3 1,0 27,4

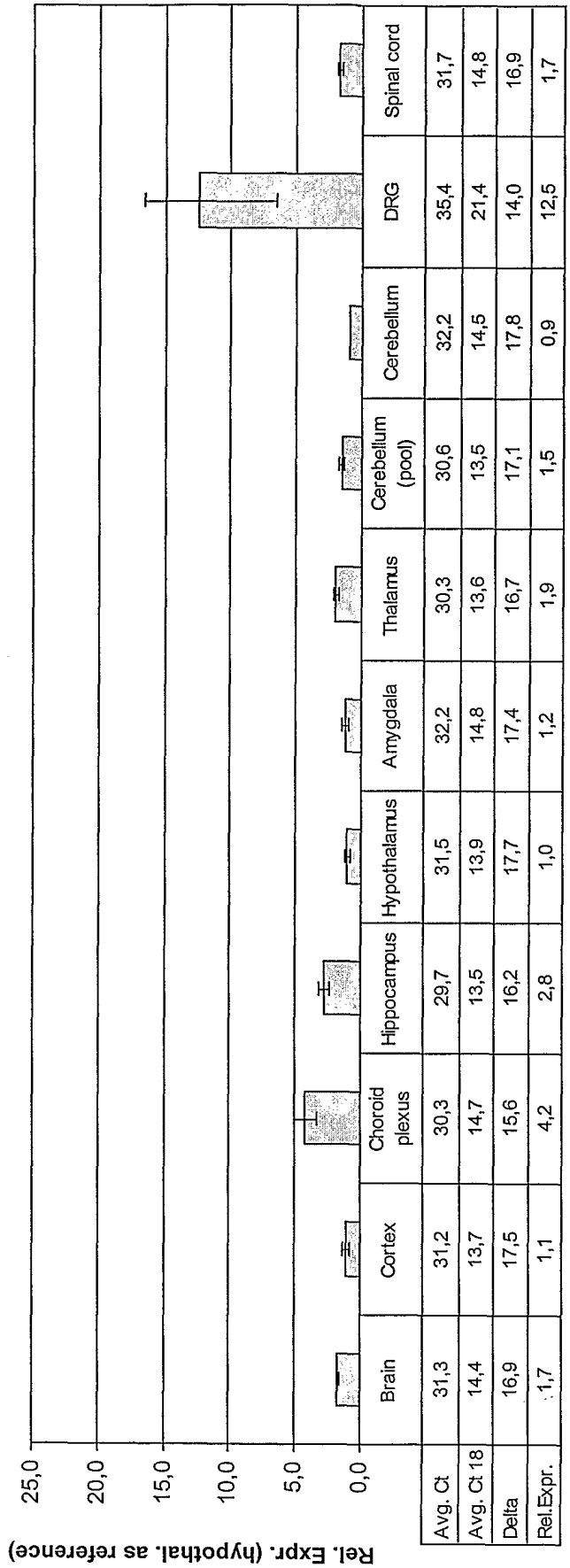
FIG. 12



	f.liver (pool)	bladder	adrenal gland (pool)	spleen (pool)	prostate (pool)	prostate	path. prostate
Avg. Ct	39,0	34,7	28,8	30,3	32,3	31,5	30,4
Avg. Ct 18S	13,0	13,9	13,6	14,2	13,8	12,1	12,0
Delta		20,8	15,2	16,1	18,6	19,5	18,4
Rel.Expr.	0,0	4,7	231,8	123,9	22,6	12,1	25,1

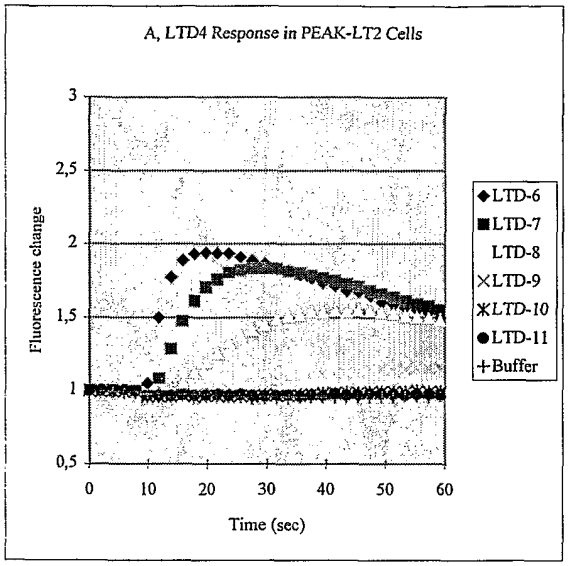
FIG. 13

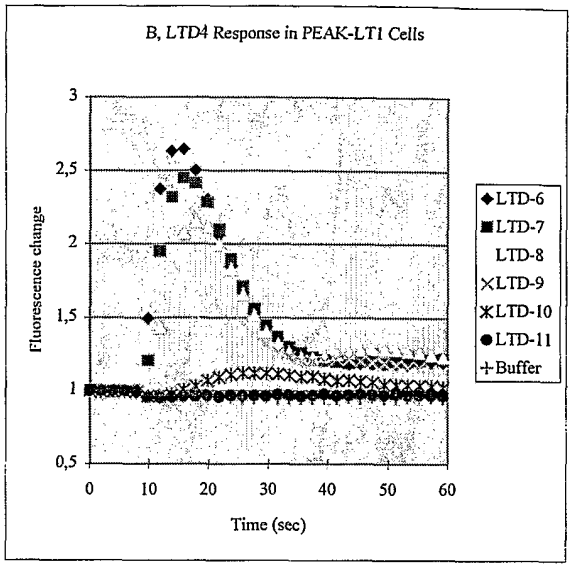
FIG. 14

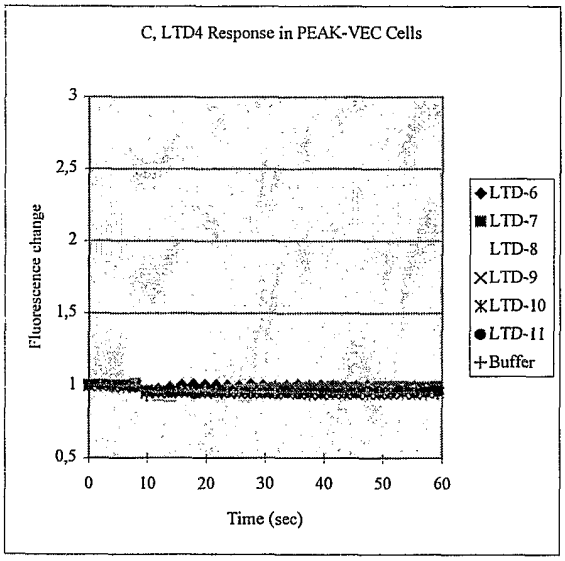


	Brain	Cortex	Choroid plexus	Hippocampus	Hypothalamus	Amygdala	Thalamus	Cerebellum (pool)	Cerebellum	DRG	Spinal cord
Avg. Ct	31,3	31,2	30,3	29,7	31,5	32,2	30,3	30,6	32,2	35,4	31,7
Avg. Ct 18	14,4	13,7	14,7	13,5	13,9	14,8	13,6	13,5	14,5	21,4	14,8
Delta	16,9	17,5	15,6	16,2	17,7	17,4	16,7	17,1	17,8	14,0	16,9
Rel.Expr.	1,7	1,1	4,2	2,8	1,0	1,2	1,9	1,5	0,9	12,5	1,7

FIG. 15


A, LTD4 Response in PEAK-LT2 Cells
Fluorescence change
Time (sec)
LTD-6
LTD-7
LTD-8
LTD-9
LTD-10
LTD-11
Buffer


B, LTD4 Response in PEAK-LT1 Cells
Fluorescence change
Time (sec)
LTD-6
LTD-7
LTD-8
LTD-9
LTD-10
LTD-11
Buffer


C, LTD4 Response in PEAK-VEC Cells
Fluorescence change
Time (sec)
LTD-6
LTD-7
LTD-8
LTD-9
LTD-10
LTD-11
Buffer

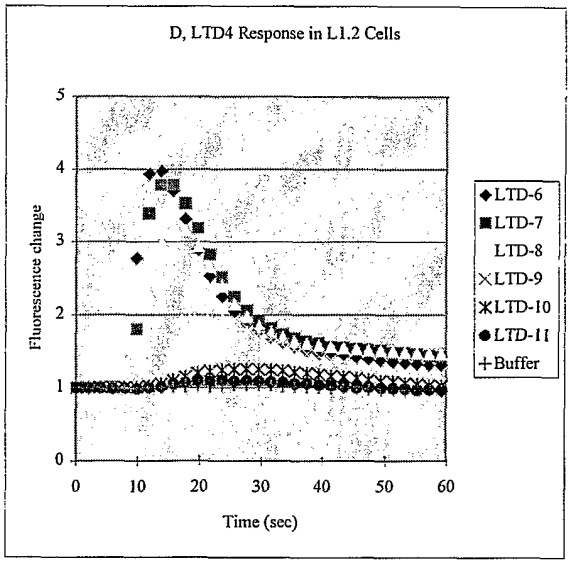
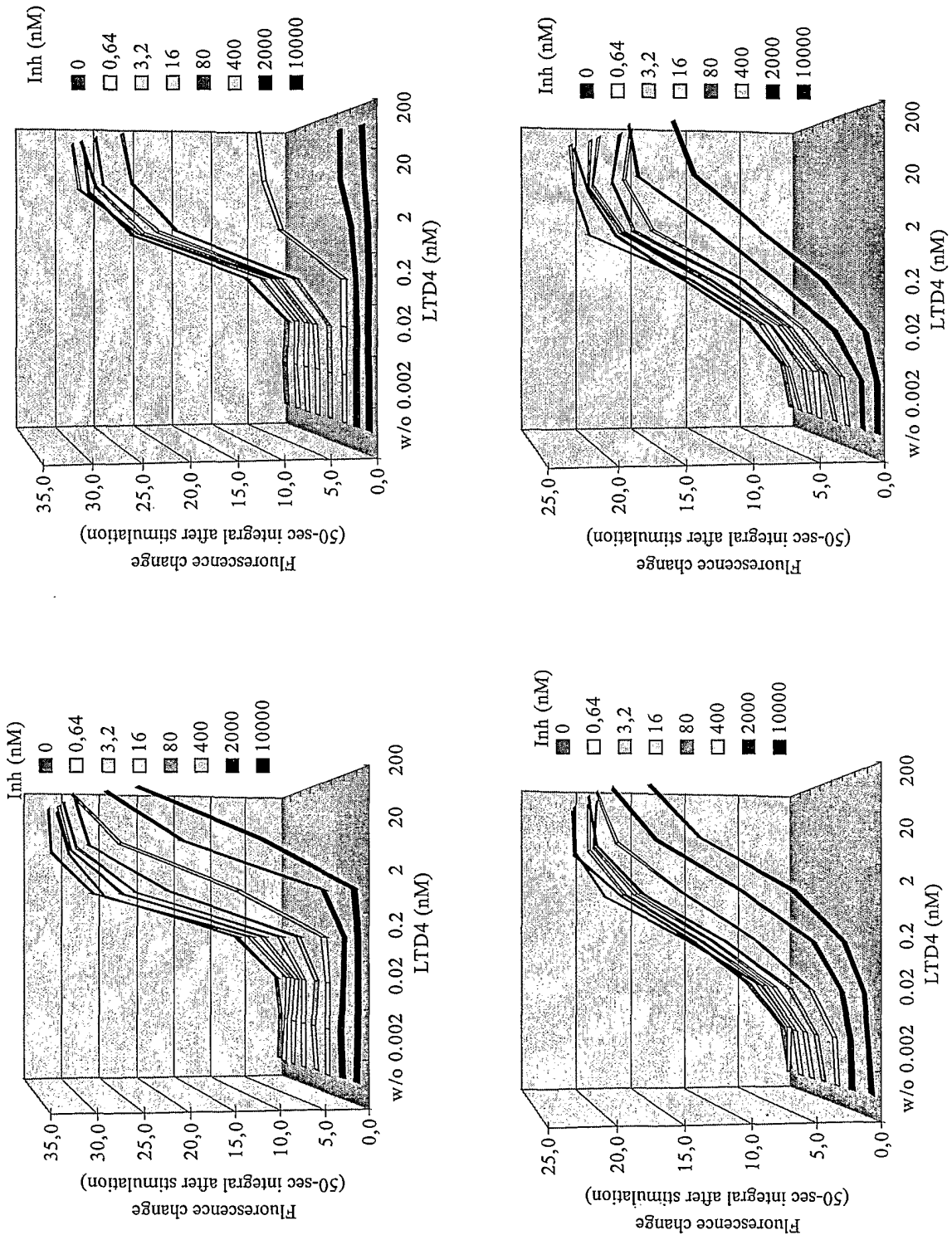

D, LTD4 Response in L1.2 Cells
Fluorescence change
Time (sec)
LTD-6
LTD-7
LTD-8
LTD-9
LTD-10
LTD-11
Buffer

FIG. 16a


Inh (nM)
0
0,64
3,2
16
80
400
2000
10000
Fluorescence change
(50-sec integral after stimulation)
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
w/o 0.002 0.02 0.2 2 20 200
LTD4 (nM)

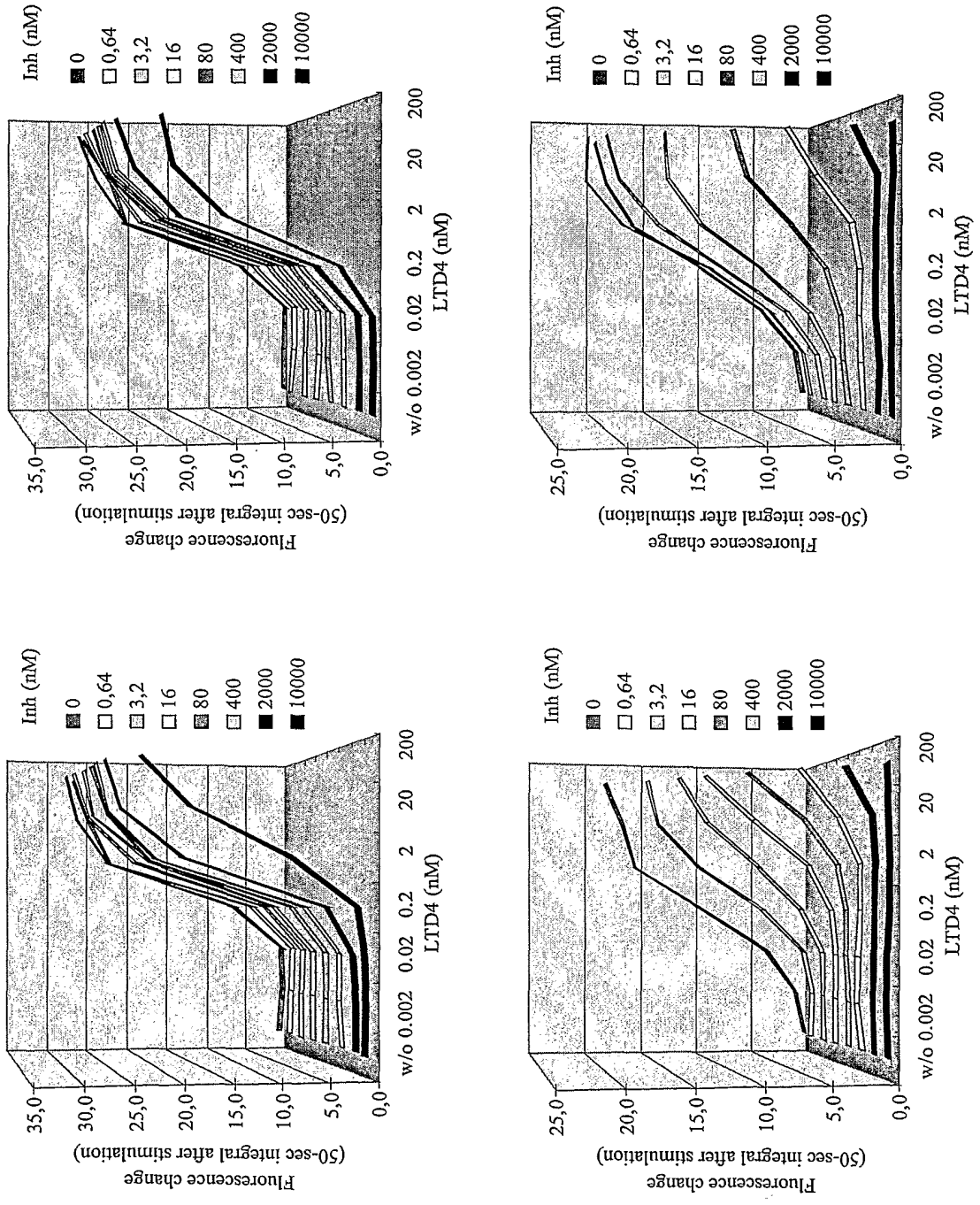

Inh (nM)
0
0,64
3,2
16
80
400
2000
10000
Fluorescence change
(50-sec integral after stimulation)
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
w/o 0.002 0.02 0.2 2 20 200
LTD4 (nM)

FIG. 16b

FIG. 17

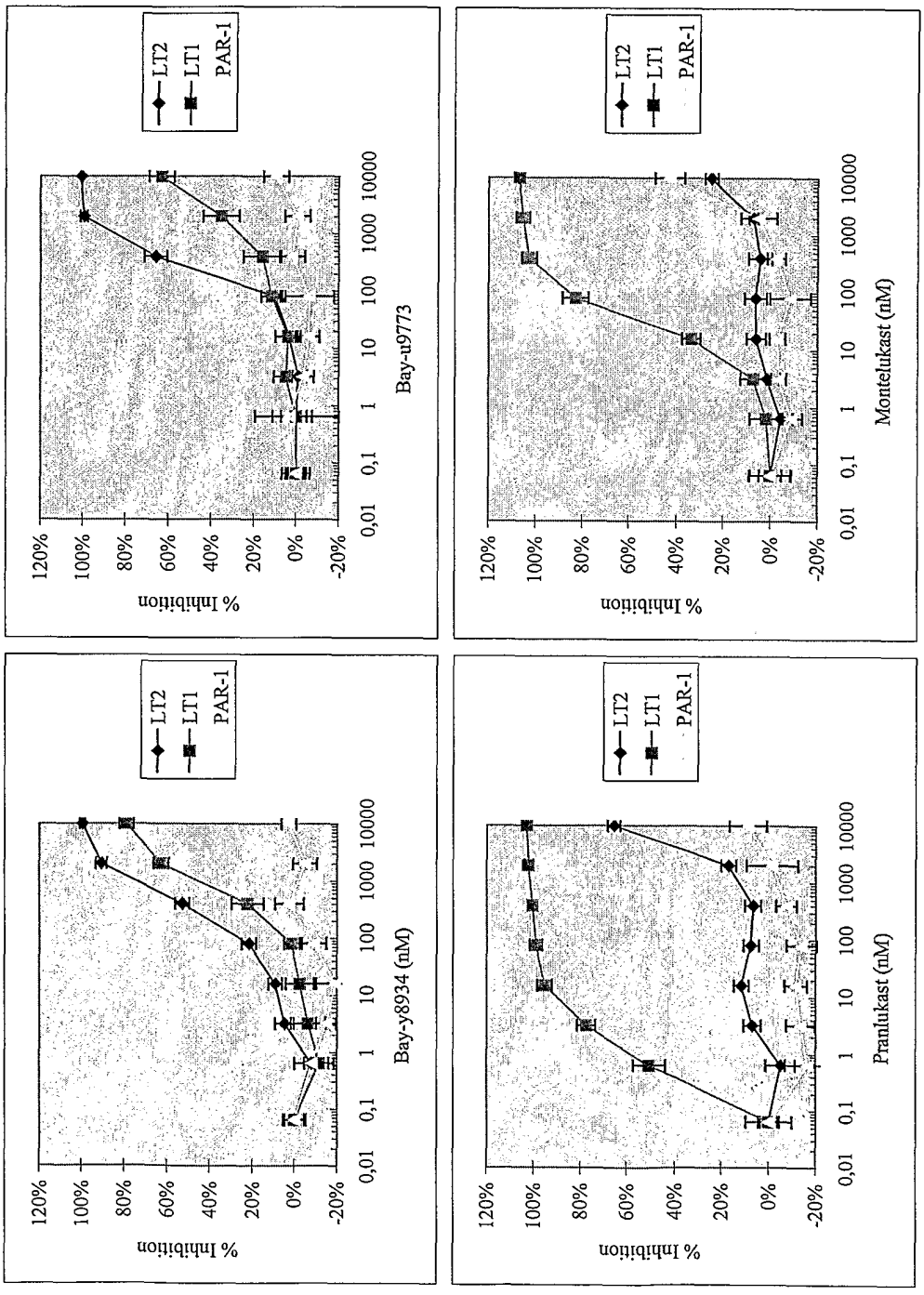
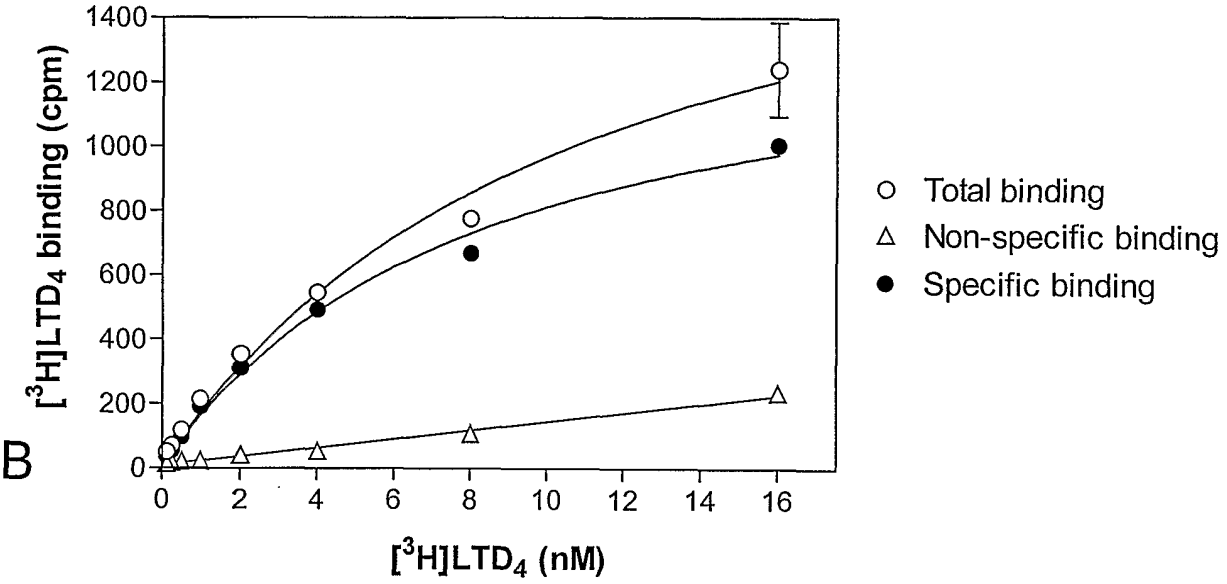
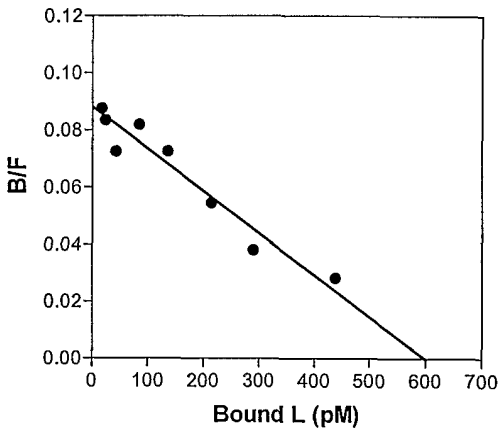

LT2
LT1
PAR-1
% Inhibition
120%
100%
80%
60%
40%
20%
0%
-20%
0,01
0,1
1
10
100
1000
10000
Bay-y8934 (nM)
Bay-u9773
Montelukast (nM)
Pranlukast (nM)

FIG. 18

A

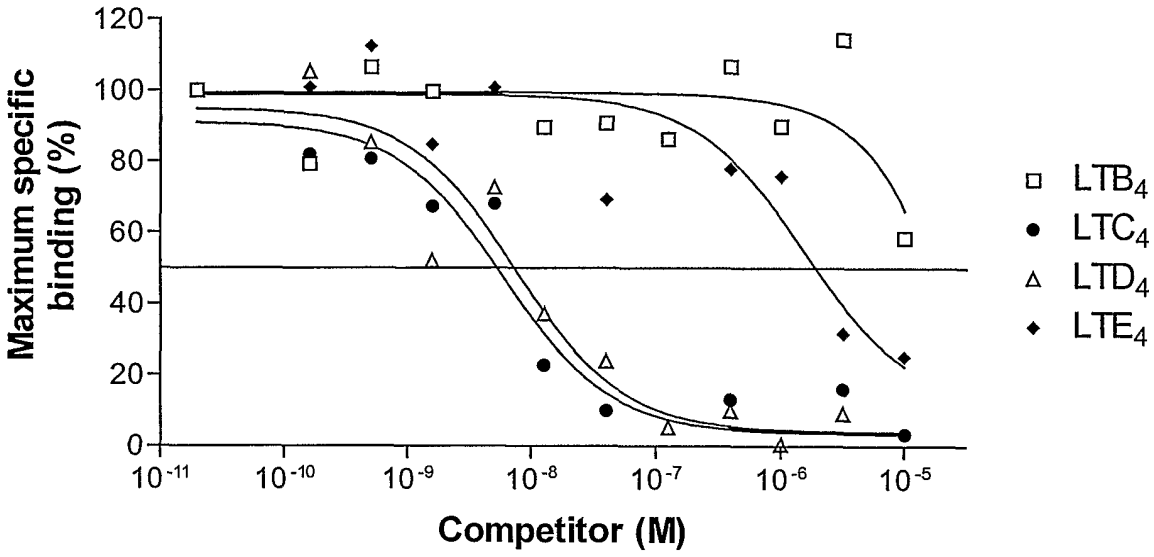


B



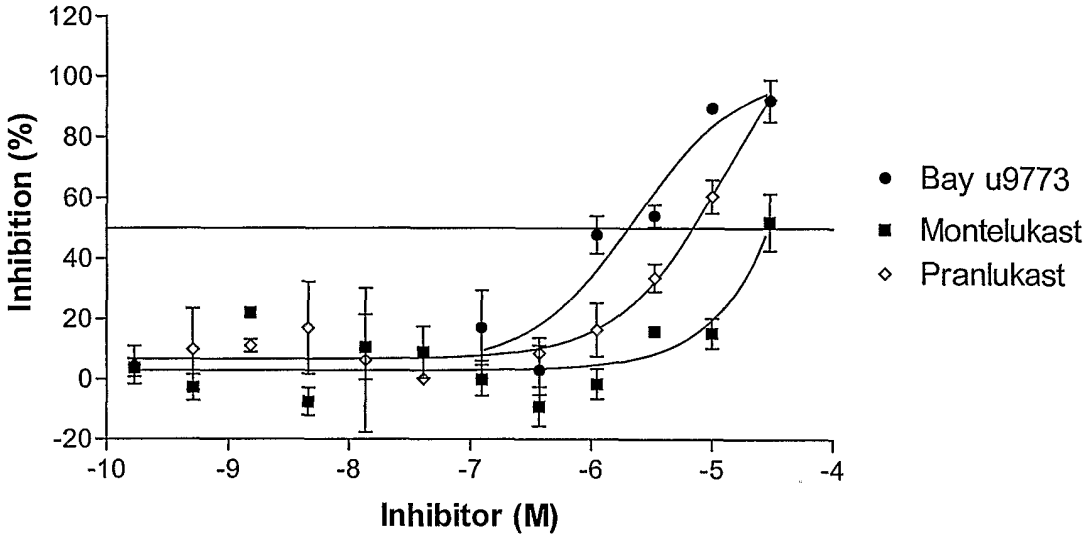
A. Saturation binding of [^{3}H]LTD$_4$ to the membrane of a CysLT2-expressing stable transfectant.

FIG. 19



Competition for [^{3}H]LTD$_4$-specific binding to CysLT2.

FIG. 20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100
80
60
40
20
0
-20
Inhibition (%)
-10
-9
-8
-7
-6
-5
-4
Inhibitor (M)
Bay u9773
Montelukast
Pranlukast

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             20                  25                  30

Ile Glu Asn Phe Lys Arg Glu Phe Phe Pro Ile Val Tyr Leu Ile Ile
         35                  40                  45

Phe Phe Trp Gly Val Leu Gly Asn Gly Leu Ser Ile Tyr Val Phe Leu
     50                  55                  60

Gln Pro Tyr Lys Lys Ser Thr Ser Val Asn Val Phe Met Leu Asn Leu
 65                  70                  75                  80

Ala Ile Ser Asp Leu Leu Phe Ile Ser Thr Leu Pro Phe Arg Ala Asp
                 85                  90                  95

Tyr Tyr Leu Arg Gly Ser Asn Trp Ile Phe Gly Asp Leu Ala Cys Arg
            100                 105                 110

Ile Met Ser Tyr Ser Leu Tyr Val Asn Met Tyr Ser Ser Ile Tyr Phe
        115                 120                 125

Leu Thr Val Leu Ser Val Val Arg Phe Leu Ala Met Val His Pro Phe
    130                 135                 140

Arg Leu Leu His Val Thr Ser Ile Arg Ser Ala Trp Ile Leu Cys Gly
145                 150                 155                 160

Ile Ile Trp Ile Leu Ile Met Ala Ser Ser Ile Met Leu Leu Asp Ser
                165                 170                 175

Gly Ser Glu Gln Asn Gly Ser Val Thr Ser Cys Leu Glu Leu Asn Leu
            180                 185                 190

Tyr Lys Ile Ala Lys Leu Gln Thr Met Asn Tyr Ile Ala Leu Val Val
        195                 200                 205

Gly Cys Leu Leu Pro Phe Phe Thr Leu Ser Ile Cys Tyr Leu Leu Ile
    210                 215                 220

Ile Arg Val Leu Leu Lys Val Glu Val Pro Glu Ser Gly Leu Arg Val
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225 230 235 240

Ser His Arg Lys Ala Leu Thr Thr Ile Ile Ile Thr Leu Ile Ile Phe
245 250 255

Phe Leu Cys Phe Leu Pro Tyr His Thr Leu Arg Thr Val His Leu Thr
260 265 270

Thr Trp Lys Val Gly Leu Cys Lys Asp Arg Leu His Lys Ala Leu Val
275 280 285

Ile Thr Leu Ala Leu Ala Ala Ala Asn Ala Cys Phe Asn Pro Leu Leu
290 295 300

Tyr Tyr Phe Ala Gly Glu Asn Phe Lys Asp Arg Leu Lys Ser Ala Leu
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Ser Val Trp Leu Arg Lys Glu Thr Arg Val
340 345

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aactcaactt tcaaagaaaa gatagtattg ctccctgttt cattaaaacc tagagagatg 180
taatcagtaa gcaagaagga aaaagggaaa ttcacaaagt aacttttgt gtctgtttct 240
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gggttgtcca tatatgtttt cctgcagcct tataagaagt ccacatctgt gaacgttttc 480
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