Identification, Molecular Cloning, Expression, and Characterization of a Cysteinyl Leukotriene Receptor

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ABSTRACT

The cysteinyl leukotrienes (CysLTs) have been implicated in the pathophysiology of inflammatory disorders, in particular asthma, for which the CysLT receptor antagonists pranlukast, zafirlukast, and montelukast, have been introduced recently as novel therapeutics. Here we report on the molecular cloning, expression, localization, and pharmacological characterization of a CysLT receptor (CysLTR), which was identified by ligand fishing of orphan seven-transmembrane-spanning, G protein-coupled receptors. This receptor, expressed in human embryonic kidney (HEK)-293 cells responded selectively to the individual CysLTs, LTC4, LTD4, or LTE4, with a calcium mobilization response; the rank order potency was LTD4 (EC\textsubscript{50} = 2.5 nM) > LTC4 (EC\textsubscript{50} = 24 nM) > LTE4 (EC\textsubscript{50} = 240 nM). Evidence was provided that LTE4 is a partial agonist at this receptor. [3H]LTD4 binding and LTD4-induced calcium mobilization in HEK-293 cells expressing the CysLT receptor were potently inhibited by the structurally distinct CysLTR antagonists pranlukast, montelukast, zafirlukast, and pobilkukast; the rank order potency was pranlukast > zafirlukast > montelukast > pobilkukast. LTD4-induced calcium mobilization in HEK-293 cells expressing the CysLT receptor was not affected by pertussis toxin, and the signal appears to be the result of the release from intracellular stores. Localization studies indicate the expression of this receptor in several tissues, including human lung, human bronchus, and human peripheral blood leukocytes. The discovery of this receptor, which has characteristics of the purported CysLT\textsubscript{1} receptor subtype, should assist in the elucidation of the pathophysiological roles of the CysLTs and in the identification of additional receptor subtypes.

The cysteinyl leukotrienes (CysLTs) are lipid mediators, generated de novo from membrane-associated arachidonic acid (Samuelsson, 1983; Drazen and Austen, 1987), which have been implicated as important contributors in the pathophysiology of several inflammatory disorders, in particular asthma (Samuelsson, 1983; Drazen and Austin, 1987; Hay et al., 1995; Horwitz et al., 1998). CysLT research began about 60 years ago when Kellaway and Trethewie (1940) demonstrated in a bioassay that effluent from antigen-stimulated guinea pig lung tissue contracted gastrointestinal smooth muscle tissue. This material, designated “slow reacting substance” (SRS), was renamed “slow reacting substance of anaphylaxis” by Brocklehurst (1960). Research in the late 1970s and early 1980s led to the elucidation of the structures and synthetic pathways for the leukotrienes including the CysLTs (LTC\textsubscript{4}, LTD\textsubscript{4}, and LTE\textsubscript{4}), which were revealed to be responsible for the biological properties of slow-reacting substance of anaphylaxis (Borgeat and Samuelsson, 1979; Murphy et al., 1979; Corey et al., 1980; Samuelsson, 1983). Extensive preclinical and clinical research on the CysLTs and their antagonists in the pulmonary system culminated in the recent introduction of three potent and selective cysteinyl leukotriene receptor (CysLTR) antagonists pranlukast (Onon; SB 205312; Ono-1078) (Obata et al., 1992; Tamaoki et al., 1997), zafirlukast (ICI 204,219; Accolate) (Krell et al., 1990; Spector et al., 1994), and montelukast (MK-476; Singulair) (Jones et al., 1995; Reiss et al., 1996) for the treatment of asthma.

ABBREVIATIONS: CysLTs, cysteinyl leukotrienes; CysLTR, cysteinyl leukotriene receptor; HEK, human embryonic kidney; HEK-293-CysLTR cells, HEK-293 cells expressing the CysLT receptor; SRS, slow reacting substance; EST, expressed sequence tag; FLIPR, fluorescent imaging plate reader; PCR, polymerase chain reaction; RT, reverse transcription; DMSO, dimethyl sulfoxide; GPCR, G protein-coupled receptor.
Binding and functional studies have provided evidence that the biological effects of the CysLTRs are mediated via G protein-coupled receptors (GPCRs; Crooke et al., 1990; Metters, 1995). There is pharmacological evidence for subtypes of CysLTRs (Coleman et al., 1995; Metters, 1995), including in human lung (Labat et al., 1992; Gorenne et al., 1996); these receptors have been classified as CysLT₁ and CysLT₂ (Coleman et al., 1995). Responses produced by stimulation of CysLT₁ are sensitive to inhibition by many CysLTR antagonists from distinct structural classes, whereas those elicited by CysLT₂ activation are resistant to most CysLTR antagonists, but antagonized by Bay u9773, which is the only known mixed CysLT₁ and CysLT₂ receptor antagonist (Coleman et al., 1995).

Significant effort has been applied to pair-activating ligands with novel orphan seven-transmembrane-spanning GPCRs identified in expressed sequence tag (EST) databases. Using the “reverse pharmacology” approach (Stadel et al., 1997) we identified a receptor designated HMTMF81 (called CysLTR vide supra) that demonstrated the characteristics of a CysLTR, including selective stimulation of calcium mobilization by all the CysLTRs, LTC₄, LTD₄, and LTE₄. The GenBank accession number for HMTMF81 is AF133266, and a patent was published on October 28, 1998 (EP 0874047). Herein the identification, molecular cloning, expression, localization, and pharmacological characterization of this CysLTR is described. Note, during the review of this manuscript another group published the results of some experiments on the characterization of the same receptor (HG55, GenBank accession number AF119711; Lynch et al., 1999).

**Experimental Procedures**

**Materials.** Pranlukast, zafirlukast, montelukast, pobilukast, LTC₄, and LTD₄ were synthesized by colleagues in the Department of Medicinal Chemistry, SmithKline Beecham Pharmaceuticals (King of Prussia, PA); LTE₄ was purchased from Biomol (Plymouth Meeting, PA).

**Identification, Cloning, and Sequencing of CysLTR.** Bioinformatic or computational surveys of both public and private (collaboration with Human Genome Sciences, Rockville, MD) EST and genomic databases were used to identify numerous sequences that encode structural characteristics common to GPCRs, a superfamily of membrane-bound proteins that activate intracellular heterotrimeric G proteins. The ESTs were converted into full-length cDNAs, transiently or stably expressed in human embryonic kidney (HEK)-293 cells (American Type Culture Collection, Rockville, MD), which were grown in Eagle’s Minimum Essential medium with Earles salt supplemented with nonessential amino acids and 10% FBS. The cells were screened for Ca²⁺ mobilization responses to a variety of known CysLTR agonists using a 96-well fluorescent imaging plate reader (FLIPR; Schroeder and Neagle, 1996).

**EST computational analysis** (Adams et al., 1991) was used to identify clone HMTMF81, which was derived from a poly-inosinic/-poly-cytidylic-stimulated peripheral blood mononuclear cell cDNA library constructed and sequenced by Human Genome Sciences (Rockville, MD). The cDNA insert of HMTMF81 was 960 bp in length, lacking approximately half of the C-terminal portion of the open reading frame. The missing region of the cDNA was isolated from both human leukocyte and spleen cDNAs using the Clontech (Palo Alto, CA) Marathon cDNA amplification kit. The DNA sequences of multiple cDNA products from separate polymerase chain reactions (PCRs) were determined to confirm the fidelity of the receptor DNA sequence. The complete 1014-bp CysLTR open reading frame was subcloned into the expression vector pCDN for expression in mammalian cells; pCDN is an in-house vector that has a CMV promotor and a neomycin-resistance marker (Aiyar et al., 1994).

**Expression in HEK-293 Cells.** Transient and stable expression in HEK-293 cells was carried out using 5 μl of lipofectamine according to the manufacturer’s instructions (Life Technology, Inc., Gaithersburg, MD) with 2 μg of plasmid DNA encoding the CysLTR gene. Stable cell lines were selected in geneticin and clones were screened by LDT₄-induced Ca²⁺ mobilization in FLIPR (as described below), and the clonal cell line producing the most potent and largest Ca²⁺ response to LDT₄ (100 nM) was used for the experiments described.

**Calcium Mobilization Experiments.** Calcium mobilization studies were conducted using Fluo 3-loaded HEK-293 cells stably expressing the CysLTR and a microtiter plate-based assay, using FLIPR (Molecular Devices, Sunnyvale, CA; Schroeder and Neagle, 1996). After growing HEK-293 cells expressing the CysLTR receptor (HEK-293-CysLTR cells) to confluence and allowing them to adhere to the FLIPR microtiter plates, growth media was removed and replaced with 1 μM Fluo-3 AM fluorescent indicator dye (Molecular Probes, Eugene, OR) in Hank’s balanced salt solution with 10 mM HEPES, 200 μM CaCl₂, 0.1% BSA, and 2.5 mM probenecid. After incubation for 1 h (37°C, 5% CO₂), cells were washed three times with the same buffer. At the initiation of the experiment, fluorescence is read every 1 s for 1 min and then every 3 s for the following minute. Agonist was added after 10 s and concentration-response curves were obtained by calculating the maximal fluorescent counts above background after addition of each concentration of agonist to define the response for each agonist concentration. The EC₅₀ is the concentration of agonist producing 50% of the maximum response. For antagonist studies, the IC₅₀ was the concentration required to inhibit 50% of the response to 33 nM LDT₄.

**Binding Studies.** HEK-293 cells transiently transfected with the CysLTR were harvested and crude membranes were prepared. Competition binding studies were conducted by standard techniques with 1.5 nM [³²P]D,L,L-DHET (20–30 Ci/mmol; New England Nuclear, Boston, MA), and 200 to 250 μg of cell membrane protein. Samples were incubated in 250 μl of piperazine-N,N’-bis(2-ethanesulfonic acid) (pH, 6.5; Sigma Chemical Co., St. Louis, MO), 10 mM CaCl₂, 10 mM MgCl₂, 10 mM glycine, and 10 mM cysteine for 45 min at 25°C. Non-specific binding was determined in the presence of 1.0 μM cold LDT₄, and accounted for 40 to 45% of total binding. Membranes were captured on Whatman GF/C filters using a Brandel cell harvester, then washed and counted with 10 ml of Beckman Ready Safe (Fullerton, CA); the radioactivity was quantitated by scintillation spectrometry. The IC₅₀ is the concentration of antagonist required to inhibit 50% of the specific binding.

**RNA Purification, PCR, and Northern Blot Analysis.** For reverse transcription (RT)-PCR studies, total RNA was extracted from tissues and cells using RNAzol B (Tel-Test, Inc., Friendswood, TX) and purified according to manufacturer’s instructions. RT-PCR was carried out on DNase-treated total RNA samples using a commercial RNA PCR kit (PE Applied Biosystems, Foster City, CA) on a Hybaid Thermocycler (Teddington, Middlesex, UK). CysLTR oligonucleotide primers were as follows: Upstream primer, 753–769, 5’-TTAGACAGTTCAGTATTTTTCCGA-3’, and a Southern blot of these results is shown in Fig. 1A. mRNA expression was calculated by hybridization to a α⁴²PdCTP-labeled (Amersham) 1100-bp fragment of the CysLTR cDNA probe. Blots were hybridized with probe at 68°C for 18 h with ExpressHyb buffer. The blots were washed four times for 15 min each at room temperature in 2× SSC, 0.05% SDS followed by four washes of 15 min each at 55°C in 0.1× SSC, 0.1% SDS. The blot was exposed at room temperature for 2 days in a phosphor cassette. Bands were visualized on a phosphor imager. Blots were stripped with hot 0.5% SDS and reprobed with β-actin cDNA probe (Clontech).
TaqMan mRNA Profiles. Poly A + RNA from multiple tissues of four different individuals (two males, two females, except prostate) was prepared, (Biochain, San Leandro, CA; Clontech, Palo Alto, CA; Invitrogen, Leek, the Netherlands; Analytical Biological Services, Wilmington, DE) or donated (Netherlands Brain Bank, Amsterdam, the Netherlands) and 1 μg of each RNA was reverse transcribed using random priming according to the Superscript II RT manufacturer's instructions (Life Technologies, Paisley, Scotland). The cDNA prepared was diluted to produce 1,000 identical microtiter plates, each containing the cDNA produced from 1 ng RNA from each tissue, and TaqMan PCR (P.E. Biosystems, Warrington, UK) was performed to detect either CysLTR or housekeeping genes. Quantitation of mRNA-derived TaqMan signal was achieved using known plasmid/genomic DNA standards included in each run on an ABI 7700 Sequence Detector (P.E. Biosystems). The negligible level of genomic DNA contamination in the RNA samples was determined by the same procedure, omitting reverse transcriptase, and the levels were subtracted from the reverse transcribed samples. CysLTR gene-specific reagents: forward primer 5'-TCCTTAAATCCTGCCTGCTTCGCA-TCACAGG-3', reverse primer 5'-GAAGAACCTGGGCTTCTACATTTTACTGC-3', TaqMan probe 5'-CCCTCAGTTGCTGCTTCGCA-3'.

Results

Identification and Molecular Characteristics of Cys-LTR. Screening of numerous orphan GPCRs for Ca2+ mobilization responses to hundreds of known and proposed GPCR ligands using FLIPR identified a transiently transfected receptor cell line that responded specifically to 1 μM LTC4 or LTD4. In parallel experiments, cells transfected with other receptors or with the empty vector did not respond to either LTC4 or LTD4. The full-length cDNA for this receptor, HMTMF81 (Cys-LTR), which was stably transfected into HEK-293 cells, had a 1579-bp sequence and encoded a protein of 337 amino acid residues (Fig. 1). Analysis of the DNA sequence by FASTA and BLAST algorithms indicated homol-
ogy of this polypeptide sequence to the seven-transmembrane-spanning GPCRs. In addition, hydrophobicity plot analysis using Lasergene Protean software showed the existence of seven hydrophobic regions, each containing approximately 20 to 30 amino acids, which are likely to represent the membrane-spanning domains found among the GPCRs. This CysLTR has the highest homology to the F_{2y} purinoceptor (31% amino acid identity) and possesses 28% identity to LTE4 (0.1 nM–10 μM) with marked, transient, concentration-dependent elevations of intracellular Ca^{2+}. The relative potencies were LTD4 > LTC4 > LTE4, with respective EC_{50} values of 2.5, 24, and 240 nM (n = 4; Fig. 2A). LTC4 and LTD4 produced a similar maximum response (P > .05), whereas the maximum response elicited by LTE4 was only approximately 40% of that produced by LTC4 and LTD4 (P < .05). More than 900 ligands, including greater than 200 ligands known to activate GPCRs, did not elicit specific Ca^{2+} mobilization responses in HEK-293-CysLTR cells; for example, LT_{B4} (0.1 nM–3 μM), the nonCysLT, was without effect (Fig. 2A). The CysLTR was also transiently transfected into Cos 7 cells and stably transfected into CHO cells. LTD4 produced a concentration-dependent Ca^{2+} mobilization in both systems with a potency similar to that observed in HEK-293-CysLTR cells (data not shown). However, the magnitude of the maximum response to LTD4 (1 μM) in Cos 7-CysLTR and CHO-CysLTR cells was much lower (at least 10-fold) than that obtained in HEK-293-CysLTR cells (data not shown).

The structurally distinct, potent CysLT receptor antagonists pranlukast (Obata et al., 1992; Tamaoki et al., 1997), zafirlukast (Krell et al., 1990; Spector et al., 1994), montelukast (Jones et al., 1995; Reiss et al., 1996), and pobilukast (SK&F 104353; Hay et al., 1987) produced concentration-dependent inhibition of the 33 nM LTD4-induced Ca^{2+} mobilization in HEK-293-CysLTR cells, with respective IC_{50} values of 0.1, 0.26, 2.3, and 5.5 nM (n = 4; Fig. 2C).

Pharmacological Characterization of CysLTR: Signal Transduction Experiments. Pertussis toxin treatment (25 ng/ml for 18 h) of HEK-293-CysLTR cells did not affect the Ca^{2+} responses produced by LTC4, LTD4, or LTE4 (Fig. 2B). LTD4-induced responses were measured in the presence and absence of extracellular calcium; most of the response (>80%) persisted after removal of extracellular calcium (data not shown).

Pharmacological Characterization of CysLTR: Binding Experiments. Radioligand-binding studies using transiently transfected HEK-293-CysLTR cell membranes demonstrated that the binding of [3H]LTD4 was specific and high-affinity; IC_{50} for cold LTD4 = 9 ± 3 nM (n = 3). Competitive binding studies with pranlukast, zafirlukast, montelukast, and pobilukast revealed IC_{50} values of 4.9 ± 0.9, 1.8 ± 0.7, 4.9 ± 1.2, and 30 nM (n = 2–7), respectively, for inhibition of [3H]LTD4 binding (Fig. 3).

Localization and Expression of CysLTR. Sites of expression of the CysLTR were evaluated using multiple cell and tissue Northern blot hybridization and TaqMan analysis (Fig. 4). Using a 1100-bp insert of CysLTR cDNA as a probe, a mRNA species of approximately 2.8 kb was revealed by Northern blots in lung, pancreas, prostate, skeletal muscle, placenta, brain, colon, heart, spleen, kidney, peripheral blood leukocytes, liver, and small intestine (Fig. 4A). There was
little or no expression detected in ovary, thymus, and testes. There was no difference in the expression of actin between the individual samples (Fig. 4A). A key finding was the demonstration of CysLTR expression in human bronchus (Fig. 4B). In addition, the receptor was detected in U937 cells (basal and dimethyl sulfoxide (DMSO)-differentiated) and HL-60 (basal and DMSO-differentiated); in the latter cells, differentiation with DMSO produced an increase in expression of CysLTR.

To confirm the presence of this CysLTR in human lung tissue, RT-PCR analysis was performed using RNA from normal and asthmatic human lungs. The results showed that only the predicted 400-bp product was formed in both sets of tissues; no bands were observed in the –RT control samples (data not shown). Preliminary results of Northern experiments suggest no difference in expression of CysLTR in lungs from normal and asthmatic individuals (data not shown).

To extend the Northern data, TaqMan-quantitative RT-PCR analysis was performed to determine the relative levels of CysLTR mRNA between tissues using samples from four different nondiseased individuals (two males, two females, except prostate). The TaqMan data (Fig. 4C) confirm the Northern data and show the highest CysLTR mRNA level in peripheral blood leukocytes and spleen with otherwise widespread distribution, and negligible level in bone. Tissue CysLTR mRNA levels were found to be consistent across individuals (males and females) for any one tissue as indicated by the error bars, β-actin levels in the same samples are shown for comparison (Fig. 4C).

Discussion

There is pharmacological and biochemical evidence that the diverse effects of the CysLTs are mediated via an interaction with at least two receptors that belong to the superfamily of GPCRs. The current results provide information on the molecular cloning, expression, and characterization of a CysLTR, which structurally belongs to the GPCR family of receptors. The relative low homology (28% identity at the amino acid level) that this CysLTR has with the nonCysLT LTB4 suggests a relatively distant evolutionary relationship between the two known leukotriene receptors; this CysLTR has the highest homology to the F2α purinoreceptor (31% amino acid identity).

The relative potencies of the CysLTs for stimulation of calcium mobilization in HEK-293-CysLTR cells (LTD4 > LTC4 > LTE4) are similar to that observed in functional studies in other cells and tissues, including human pulmonary tissues (Mucitelli et al., 1987; Labat et al., 1992). Furthermore, as in the present experiment in HEK-293-CysLTR cells indicating that LTE4 is a partial agonist for eliciting calcium responses in these cells, previous studies in DMSO-differentiated human U937 cells (Saussy et al., 1989), human bronchus (Mucitelli et al., 1987), and sheep trachea (Mong et al., 1989) also have provided evidence that LTE4 has lower intrinsic activity relative to LTC4 and LTD4.

Additional studies are required to determine unequivocally if this CysLT receptor corresponds to either of the previously described CysLT1 or CysLT2 receptors, which were classified based on a comparison of the rank order potency of agonists and antagonists in various tissues from different species (Coleman et al., 1995). Pharmacologically, the CysLTR described herein has aspects of its profile, including agonist and antagonist potencies and relative agonist efficacies, which are similar in several respects to that of the CysLT1 receptor, rather than the CysLT2 receptor. For example, contraction of smooth muscles mediated by activation of CysLT1 receptors (e.g., in human bronchus) are, like [3H]LTD4 binding and LTD4-induced Ca2+ mobilization in HEK-293-CysLTR cells, potently inhibited by the various receptor antagonists used in this study (Hay et al., 1987; Krell et al., 1990; Jones et al., 1995; Metters, 1995). In contrast, responses produced by stimulation of the CysLT2 receptor are not sensitive to these compounds (Labat et al., 1992; Coleman et al., 1995; Goren et al., 1996). However, there is recent information that the proliferative effects of LTD4 in human cultured tracheal smooth muscle cells may be mediated by stimulation of an atypical CysLTR that does not fit into the current classification (CysLT1 and CysLT2) (Panettieri et al., 1998).

The lack of effect of pertussis toxin on CysLT-induced functional responses suggests that the G protein involved in transfected cells is of the Gq/11 rather than the Gi/0 class. The LTD4-induced Ca2+ response of the endogenous receptor expressed in differentiated human U937 cells is partially blocked by pertussis toxin, suggesting that this receptor is coupled to pertussis toxin-sensitive and -insensitive G proteins (Saussy et al., 1989). The Ca2+ response resulting from activation of the cloned LTβ receptor expressed in CHO cells was minimally inhibited by pertussis toxin (Yokomizo et al., 1997), whereas the LTβ4-induced response in leukocytes is pertussis toxin-sensitive (Powell et al., 1996). The LTD4-induced Ca2+ response in stable HEK-293-CysLTR cells was minimally affected by removal of extracellular Ca2+, indicating that the response was the result of release from intracellular stores. This is different from the Ca2+ response in differentiated U937 cells where most of the Ca2+ response was lost when extracellular Ca2+ was removed (Saussy et al., 1989). Differences in the G proteins expressed in transfected cells compared with cells endogenously expressing the recep-

![Figure 3](https://molpharm.aspetjournals.org/doi/10.1189/j.1525-1570.1995.1995.issue-1)

**Figure 3.** Effects of the CysLT receptor antagonists pranlukast, zafirlukast, montelukast, and pobilukast on competitive binding of [3H]-LTD4 in membranes of transiently expressed HEK-293-CysLTR cells. Results for pranlukast (●), zafirlukast (□), montelukast (○), and pobilukast (■) are presented as percent specific binding and are given as the mean of four to six experiments run with duplicate samples. The S.E.M.s for the individual points were <5%; for the sake of clarity the S.E.M.s were omitted from the graphs.
tors could account for these different sensitivities to pertussis toxin and extracellular calcium.

The presence of the CysLTR in human lung, including bronchus, agrees with previous functional studies and supports the convincing evidence from preclinical and clinical studies that the CysLTs play an important role in asthma pathogenesis. Although our preliminary assessment using RT-PCR suggested no alterations in the level of expression of the CysLTR in lungs from asthmatic versus nonasthmatic individuals, additional studies, in particular at the protein level, are required to determine whether alterations in the CysLTR expression occur in diseases of the lung and other tissues. The strong expression of the receptor on PBLs suggests that the CysLTs may have an impact on the function of T-cells, which are thought to be key cells in a variety of immune and inflammatory disorders. Furthermore, the localization of this CysLTR on U937 cell suggests that this is the endogenous receptor on this cell line, which has been used as a cellular system to identify and evaluate CysLTR antagonists; however, it does not preclude the existence of additional CysLTRs. Additional investigation is required to determine whether the presence of the receptor in other tissues has any pathophysiological significance.

During the review of this manuscript there was a published report outlining the characterization of a CysLTR, which was classified as a CysLT₁ receptor (Lynch et al., 1999; GenBank accession number, AF 119711). The sequence of the gene described in that publication is identical with HMTMF81 discussed herein, and similar findings were obtained in pharmacological and localization experiments in the two separate studies. For example, the absolute and relative potencies of pranlukast, zafirlukast, and montelukast for inhibition of the binding of [³H]LTD₄ to the expressed CysLTR were essentially the same. Furthermore, the Northern analysis profiles were similar in both studies, with high RNA expression in PBLs and spleen, and detection of lower level expression in several other tissues.

In summary, the results of the present functional, binding, and localization studies provide evidence that the CysLTR described in this publication corresponds in many respects to the CysLT₁ receptor, which is responsible for the contractile responses to the CysLTs in several mammalian airway tis-

![Fig. 4. Northern blot (A, B) and TaqMan (C) analysis of CysLTR in human tissues and cell lines. These studies were performed using human multiple tissue blots purchased from Clontech (A) and various in-house cell lines and human bronchus (B) as described in Experimental Procedures. A and B, size of the transcripts are indicated on the right. PBLs, peripheral blood leukocytes; diff., DMSO differentiated. C, TaqMan-quantitative RT-PCR analysis of mRNA levels in human tissues. The cDNA from the reverse transcription of 1 ng poly A⁺ RNA from multiple tissues for four different nondiseased individuals was assessed for its CysLTR and housekeeper genes (β-actin shown) using TaqMan PCR. Data are presented as the mean ± S.E.M. of four individuals' mRNA levels for each tissue, other than the intestine, which is an equal pool of one individual's small and another individual's large intestine.](image-url)
sues, including human bronchus. It is anticipated that the discovery of the described CysLTR may lead to the identification of additional CysLTR subtypes, other possible therapeutic opportunities for CysLTR antagonists, and a renaissance in CysLT research.

References


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