Identification in mice of two isoforms of the cysteinyl leukotriene 1 receptor that result from alternative splicing

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Two classes of human G protein-coupled receptors, cysteinyl leukotriene 1 ($CysLT_1$) and $CysLT_2$ receptors, recently have been characterized and cloned. Because the $CysLT_1$ receptor blockers are effective in treating human bronchial asthma and the mouse is often used to model human diseases, we isolated the mouse $CysLT_1$ receptor from a mouse lung cDNA library and found two isoforms. A short isoform cDNA containing two exons encodes a polypeptide of 339 aa with 87.3% amino acid identity to the human $CysLT_1$ receptor. A long isoform has two additional exons and an in-frame upstream start codon resulting in a 13-aa extension at the N terminus. Northern blot analysis revealed that the mouse $CysLT_1$ receptor mRNA is expressed in lung and skin; and reverse transcription–PCR showed wide expression of the long isoform with the strongest presence in lung and skin. The gene for the mouse $CysLT_1$ receptor was mapped to band XD. Leukotriene (LT) D_4 induced intracellular calcium mobilization in Chinese hamster ovary cells stably expressing either isoform of the mouse $CysLT_1$ receptor cDNA. This agonist effect of LTD_4 was fully inhibited by the $CysLT_1$ receptor antagonist, MK-571. Microsomal membranes from each transformant showed a single class of binding sites for $[^3H]LTD_4$; and the binding was blocked by unlabeled LTs, with the rank order of affinities being $LTD_4 >> LTE_4 = LTC_4 >> LTB_4$. Thus, the dominant mouse isoform with the N-terminal amino acid extension encoded by an additional exon has the same ligand response profile as the spliced form and the human receptor.

The cysteinyl leukotriene (LT) C_4 is synthesized by limited cell types, such as mast cells, basophils, eosinophils, and macrophages (1, 2). These cells express LTC_4 synthase, which conjugates reduced glutathione to an epoxide metabolite of arachidonic acid, LTA_4 (3, 4). After carrier-mediated export (5), the sequential cleavage of glutamic acid and glycine from the glutathione moiety of LTC_4 yields LTD_4 and LTE_4 (6, 7), respectively. Neutrophils, which lack LTC_4 synthase but express LTA_4 hydrolase, make a dihydroxy leukotriene, LTB_4, which is a potent chemotactic agent (8).

Pharmacologic analyses of the smooth muscle contractile activity of the cysteinyl leukotrienes (CysLTs) in different species provided evidence for two classes of receptors, designated $CysLT_1$ and $CysLT_2$ receptors (9, 10). The human $CysLT_1$ (11, 12) and $CysLT_2$ (13–15) receptors have been cloned and characterized, revealing only 38% amino acid identity. Both receptors are seven-transmembrane, G protein-coupled. The rank order of affinities of the LTs for the $CysLT_1$ and $CysLT_2$ receptors are $LTD_4 \gg LTC_4 > LTE_4 \gg LTB_4$ and $LTD_4 = LTC_4 \gg LTE_4 \gg LTB_4$, respectively. The genes for the human $CysLT_1$ and $CysLT_2$ receptors were mapped to chromosomes Xq13-q21 (11) and 13q14 (13), respectively. The $CysLT_1$ receptor is expressed in airway smooth muscle cells, tissue macrophages, monocytes, and eosinophils (11); and the $CysLT_2$ receptor is prominently expressed in lung macrophages, airway smooth muscle, cardiac Purkinje cells, adrenal medulla cells, peripheral blood leukocytes, and brain (13).

The contribution of the CysLT receptors to bronchial asthma has been established by the therapeutic efficacy of biosynthetic inhibitors (16) and selective $CysLT_1$ receptor blockers (17). Ovalbumin sensitization and aerosol challenge in mice elicits LTB_4 and LTC_4 release into bronchoalveolar lavage (BAL) fluid, eosinophilia of the mucosa and the BAL fluid, and increased airways reactivity to methacholine (18, 19). Although the CysLTs are not established bronchoconstrictors in mice (20, 21), a $CysLT_1$ receptor selective antagonist, MK-571, recently was shown to inhibit eosinophilia, bronchial hyperreactivity, and microvascular leakage in this model; this finding implies a contribution by CysLTs (22). Furthermore, in LTA_4 hydrolase gene-disrupted mice subjected to zymosan A-induced peritonitis, neutrophil recruitment was decreased, but protein extravasation because of increased vascular permeability in the peritoneal cavity was substantial (23). These findings are consistent with the measured absence of LTB_4 and increased CysLT generation attributed to shunting of LTA_4 to LTC_4 synthase. In addition, the CysLTs have been shown to directly increase venular permeability with edema formation at the administration site in mice (24, 25).

In this study, we have cloned and characterized the cDNA for the mouse $CysLT_1$ receptor, and determined the gene structure and the chromosomal localization. In apparent contrast to the human $CysLT_1$ receptor gene reported to contain two exons providing a single transcript (11, 12) (GenBank accession no. AC021992), there are two isoforms of the mouse $CysLT_1$ receptor cDNA that result from alternative splicing from a gene with four exons.

Materials and Methods

Cloning of the Mouse Gene for the $CysLT_1$ Receptor. To isolate the mouse $CysLT_1$ receptor cDNA, we used a human $CysLT_1$ receptor cDNA fragment as a probe. This fragment was obtained by PCR of a human lung cDNA library (Takara Shuzo, Otsu, Japan) with a T7 primer, 5′-TAATACGACTCACTATAGGG-3′, and a human $CysLT_1$ receptor-specific antisense primer, 5′-GGCCATTAGAAATGGAGAACTGGT-3′ (nucleotides +483 to +460; +1 corresponds to the A nucleotide of the ATG start codon) with *Pfu* DNA polymerase (Stratagene). The amplified fragment was sequenced and shown to contain 364 bp of 5′-noncoding nucleotides and 483 bp of coding nucleotides of the human $CysLT_1$ receptor. With this 847-bp fragment used as a ^{32}P-labeled probe, 1×10^6 plaque-forming units of a λFIXII 129Sv mouse genomic library (Stratagene) were screened by

Abbreviations: LT, leukotriene; CysLT, cysteinyl leukotriene; RT, reverse transcription; RACE, rapid amplification of cDNA ends; CHO, Chinese hamster ovary.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AF329271 and AF329272).

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plaque hybridization, and one clone containing a 10.5-kb genomic DNA fragment was isolated. Phage DNA was isolated from the positive plaque, digested with restriction enzymes, resolved by gel electrophoresis, and transferred to a nylon membrane. Genomic restriction fragments that hybridized with the ^{32}P-labeled 847-bp probe were subcloned into a pBluescriptII vector for DNA sequencing with fluorescence dye-labeled dideoxynucleotides as terminators.

cDNA Cloning of the Mouse CysLT$_1$ Receptor. A cDNA library was generated by reverse transcription (RT) of the poly(A)$^+$ RNA isolated from C57BL/6 mouse lung with an oligo(dT) primer and avian myeloblastosis virus reverse transcriptase (CLONTECH) followed by T4 DNA polymerase. The resulting double-stranded cDNAs were ligated to an adaptor cassette at both the 5′ and the 3′ ends with the Marathon cDNA Amplification Kit (CLONTECH). The 3′ and 5′ rapid amplification of cDNA ends (RACE) were performed by PCR with an adaptor primer (CLONTECH) and mouse CysLT$_1$ receptor gene-specific primers under the condition 94°C for 30 s, 65°C for 30 s, 72°C for 1 min, 35 cycles. The amplified fragments were ligated into a pCR-Script vector (Stratagene), and eight different clones from each RACE were sequenced. Full-length cDNA was then generated by PCR of the same cDNA library with primers derived from nucleotide sequences at the 5′ and the 3′ ends of the CysLT$_1$ receptor cDNA obtained through 3′ and 5′ RACE. The full-length cDNA was ligated into pCR-Script vector for sequencing.

Northern Blot and RT-PCR Analyses. Total RNA was isolated from various tissues of 8-wk-old male C57BL/6 mice with Tri-Reagent (Sigma). A 20-μg sample of the total RNA was resolved by electrophoresis on a formaldehyde-denatured gel and transferred to a nylon membrane (Gelman Sciences) with 20× SSC for 24 h. The membrane was baked at 80°C for 2 h; prehybridized at 42°C for 2 h in 5× SSC, 5× Denhardt's solution, 50% formamide, 0.2% SDS, 100 μg/ml denatured salmon sperm DNA; and then hybridized at 42°C for 16 h with the ^{32}P-labeled mouse CysLT$_1$ receptor cDNA fragment prepared with a Megaprime DNA labeling kit (Amersham Pharmacia). The blot was washed once in 0.5× SSC, 0.1% SDS at 60°C for 30 min and twice in 0.2× SSC, 0.1% SDS at 60°C for 30 min, and was then exposed to a Kodak AR film for 14 days at −80°C with an intensifying screen. The probe was stripped, and the blot was hybridized with a ^{32}P-labeled mouse glyceraldehyde-3-phosphate dehydrogenase cDNA probe.

Total RNA (100 ng) isolated as described above was used as a template for a One Step RT-PCR kit (Qiagen, Chatsworth, CA) under the condition 94°C for 30 s, 65°C for 30 s, 72°C for 1 min for 40 cycles.

Cell Culture and Stable Expression of the Mouse CysLT$_1$ Receptor. Chinese hamster ovary (CHO) cells (American Type Culture Collection) were grown in DMEM/F-12 with 10% FBS, nonessential amino acids, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified atmosphere with 5% CO_2. The cDNA for the mouse CysLT$_1$ receptor was subcloned into a pCXN vector under the control of the chicken β-actin promoter and the cytomegalovirus immediate early enhancer (26), transfected into the CHO cells by electroporation, and selected in the medium containing 800 μg/ml G418 (GIBCO/BRL). At least three independent G418-resistant clones transfected with each construct were isolated, and the expression of the construct was confirmed by Northern blot analysis. Transformants were examined for their ability to mediate ligand-specific intracellular calcium flux or [^{3}H]LTD$_4$ binding with and without competing ligands or inhibitors.

Intracellular Calcium Flux Assay. LTB$_4$, LTC$_4$, LTD$_4$, LTE$_4$, and a CysLT$_1$ receptor selective antagonist, MK-571 (27), were purchased from Cayman Chemicals (Ann Arbor, MI). One day before assay, the CHO cell stable transformants were seeded at 1×10^7 cells per 15-cm dish. The cells were collected by dispersion with 0.05% EDTA in Hanks' balanced salt solution (HBSS), resuspended in HBSS containing 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% BSA (HBSA^{2+}), and loaded with Fura-2 AM (Molecular Probes) in the presence of 2.5 mM probenecid for 30 min at 37°C. After being labeled, the cells were washed and resuspended in HBSA^{2+} (5×10^6 cells/ml). Fluorescence output was measured with excitation at 340 and 380 nm in a fluorescence spectrophotometer (Hitachi F-4500) after the cells were stimulated with LTs alone or with MK-571; and the relative ratio of fluorescence emitted at 510 nm was recorded.

[^{3}H]LTD$_4$ Binding Assay. The CHO cell transformants were harvested, washed in PBS, resuspended in PBS with 2 mM phenylmethylsulfonyl fluoride, and lysed by sonication for 5 min. The lysed cells were centrifuged at 100,000 × *g* for 30 min. The microsomal pellets were resuspended in 1 ml PBS, and a sample of each was taken for determination of protein concentration with the Bio-Rad Protein Assay Kit. [^{3}H]LTD$_4$ binding to the microsomal membranes was assayed as described (28). Briefly, 50 μg of membrane protein was incubated for 1 h at room temperature in 500 μl of 10 mM Hepes/KOH, pH 7.4, containing 20 mM $CaCl_2$, 200 pM [^{3}H]LTD$_4$ (New England Nuclear), and 20 mM L-penicillamine. Then, bound [^{3}H]LTD$_4$ was separated from free [^{3}H]LTD$_4$ by filtration through GF/C filters (Whatman) and a wash with 10 mM Hepes/KOH, pH 7.4, containing 0.01% BSA. The residual membrane-associated [^{3}H]LTD$_4$ on the filter was determined by liquid scintillation counting in 10 ml LSC mixture (Formula-989; DuPont). When LTC$_4$ was used as a competitor, 50 mM serine-borate was included to prevent the conversion from LTC$_4$ to LTD$_4$ (29). The LTD$_4$-specific binding with or without competition by a LT was determined by subtracting nonspecific binding, which was determined in the presence of 10 μM MK-571, from total binding.

Chromosomal Localization of the Mouse CysLT$_1$ Receptor Gene by Fluorescence *in Situ* Hybridization. The 10.5-kb genomic DNA fragment containing the mouse CysLT$_1$ receptor gene was labeled with digoxigenin-dUTP by nick translation. The labeled probe was combined with sheared mouse DNA and hybridized to normal metaphase chromosomes derived from mouse embryo fibroblasts in a solution containing 50% formamide, 10% dextran sulfate, and 2× SSC. Specific hybridization signals were detected when the hybridized slides were incubated in fluoresceinated anti-digoxigenin antibodies and counterstained with 4′,6-diamidino-2-phenylindole.

Results and Discussion

Structure of the cDNA and Gene for the Mouse CysLT$_1$ Receptor. We screened 10^6 plaques of a λFIXII 129Sv mouse genomic library with an 847-bp human CysLT$_1$ receptor cDNA probe obtained by PCR of a human lung cDNA library. Phage DNA from a positive plaque contained a 10.5-kb DNA insert, which on restriction digestion provided a 7-kb fragment that hybridized to the human probe. A nucleotide sequence comparison of the 7-kb fragment revealed a coding region homologous to that of the human CysLT$_1$ receptor. This region of the mouse gene encodes a 339-aa polypeptide with a calculated molecular mass of 39,160 and an 87.3% identity in amino acid sequence to that of the human CysLT$_1$ receptor (Fig. 1*A*). The secondary structure of the CysLT$_1$ receptor of the mouse and human is conserved for the seven transmembrane domains, and the six hydrophilic loops. The N terminus of the mouse CysLT$_1$ receptor (subsequently

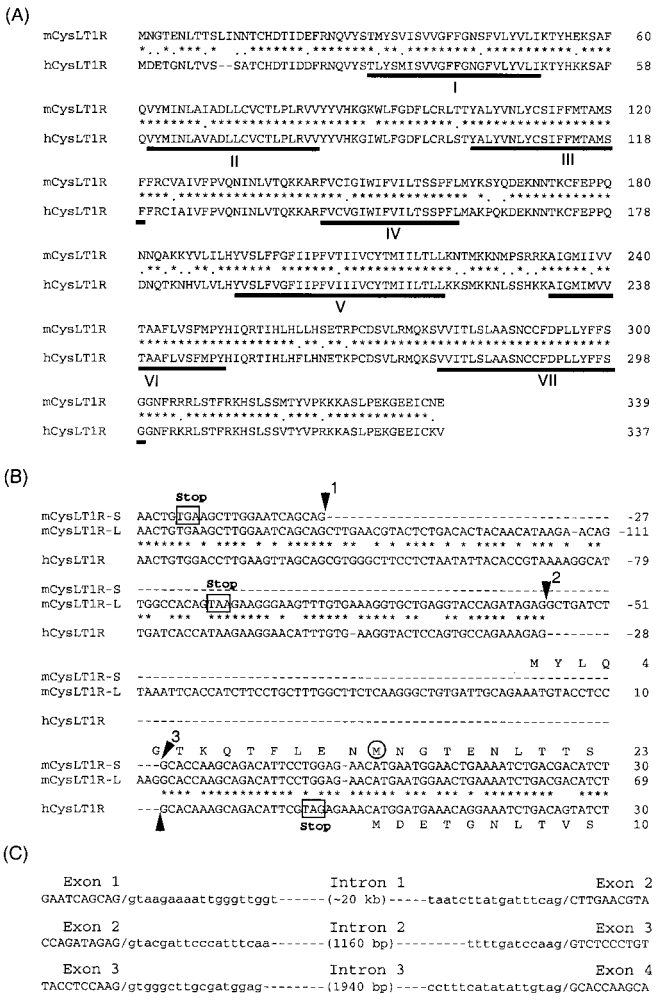

Fig. 1. Structure of the cDNA and the gene for the mouse $CysLT_1$ receptor. (*A*) Alignment of amino acid sequences of the mouse (short isoform) (mCysLT1R) and the human (hCysLT1R) $CysLT_1$ receptors. Asterisks and dots indicate identical and similar amino acids in the mouse and the human $CysLT_1$ receptors, respectively. Seven putative transmembrane domains (I–VII) are underlined. (*B*) Alignment of the nucleotide sequences of the partial 5′ noncoding and coding regions of the mouse short isoform (mCysLT1R-S), the mouse long isoform (mCysLT1R-L), and the human $CysLT_1$ receptor (hCysLT1R) cDNA. Asterisks indicate identical nucleotides in the mouse and the human. The exon/intron junctions in the mouse and the human (11) (GenBank accession no. AC021992) $CysLT_1$ receptor genes are indicated by numbered arrowheads. In-frame stop codons are shown by open boxes. The start methionine in the short isoform of the mouse cDNA is circled. For the long isoform cDNA, exon I is 5′ of arrow 1, exon II is between arrows 1 and 2, exon III is between arrows 2 and 3, and exon IV is 3′ of arrow 3. Note that the hCysLT1R is missing the equivalent of mouse exon III. (*C*) Boundary sequence of the four exons and the sizes of the three introns of the mCysLT1R-L.

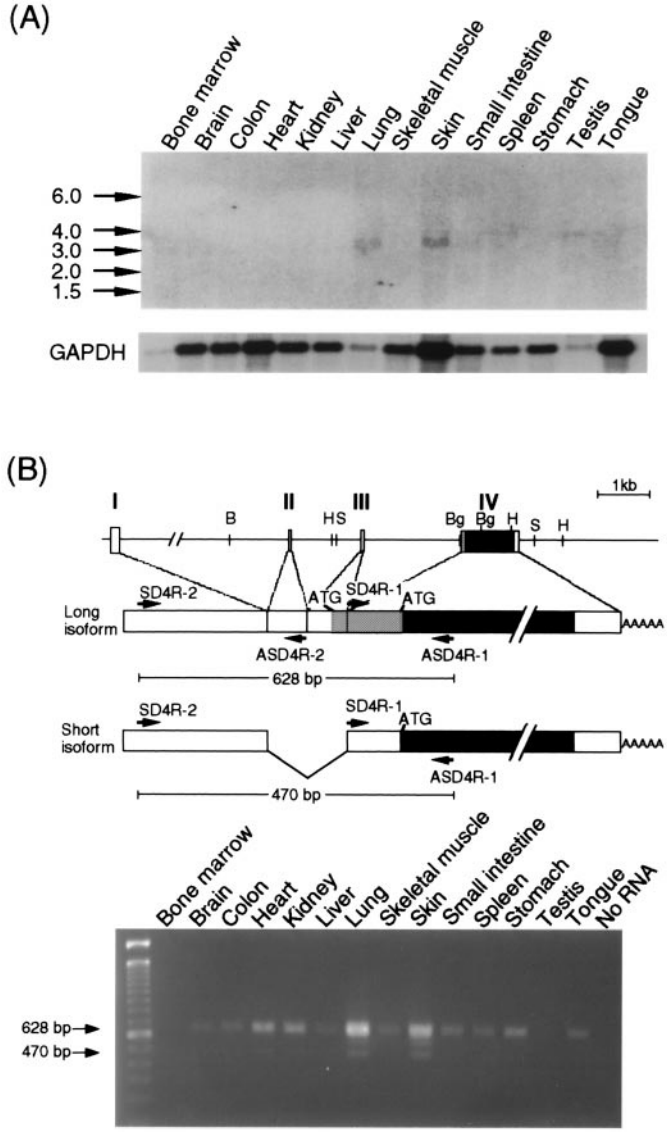

Fig. 2. Expression of the $CysLT_1$ receptor mRNA in mouse tissues. (*A*) Northern blot analysis with total RNA of C57BL/6 mouse tissues. The upper and lower panels show hybridization with the full-length cDNA for the mouse $CysLT_1$ receptor and a 450-bp fragment of the mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA, respectively. (*B*) RT-PCR analysis with 100 ng of total RNA from mouse tissues and primers common to both isoforms of the mouse $CysLT_1$ receptor. The primers are identified by arrows in the schematic diagram (*Upper*) relating the long and short isoforms to the genomic organization. The exons are shown as boxes with the common coding region in black and the N-terminal extended coding region in the long isoform in gray. Restriction sites: B, *Bam*HI; H, *Hin*dIII; S, *Sac*I; Bg, *Bgl*II. The expected size of the products is noted both in the schematic diagram and at the left of the ethidium bromide-stained gel (*Lower*).

designated short isoform) is two amino acid residues longer than the human $CysLT_1$ receptor.

To determine the genomic organization of the mouse $CysLT_1$ receptor gene, 3′ and 5′ RACE were performed by PCR on C57BL/6 mouse lung mRNA with an adaptor primer and mouse $CysLT_1$ receptor gene-specific primers, designated SD4R-1, for the 5′-GCACCAAGCAGACATTCCTGGAGA-3′ oligonucleotide (−25 to −37; +1 corresponds to the A nucleotide of the ATG start codon) and ASD4R-1 for the 5′-AATCATG-TATACTTGGAAGGCTGA-3′ oligonucleotide (+195 to +177), respectively. The amplified fragments were ligated into a pCR-Script vector, and eight different clones from each RACE were sequenced to obtain the nucleotide sequence of the 5′ untranslated region of the cDNAs. The alignment of the genomic sequence with that of the RACE products revealed that the mouse $CysLT_1$ receptor gene is comprised of four exons and three introns. One transcript (short isoform) contains exons I and IV, and the other transcript (long isoform) contains exons I, II, III, and IV. Importantly, exon III contains an in-frame start codon (ATG) that is 39 bp upstream of the ATG translation start site reported for the human $CysLT_1$ receptor and thus encodes a polypeptide with a 13-aa extension at the N terminus with 352 deduced amino acid residues and a calculated mass of 40,715.

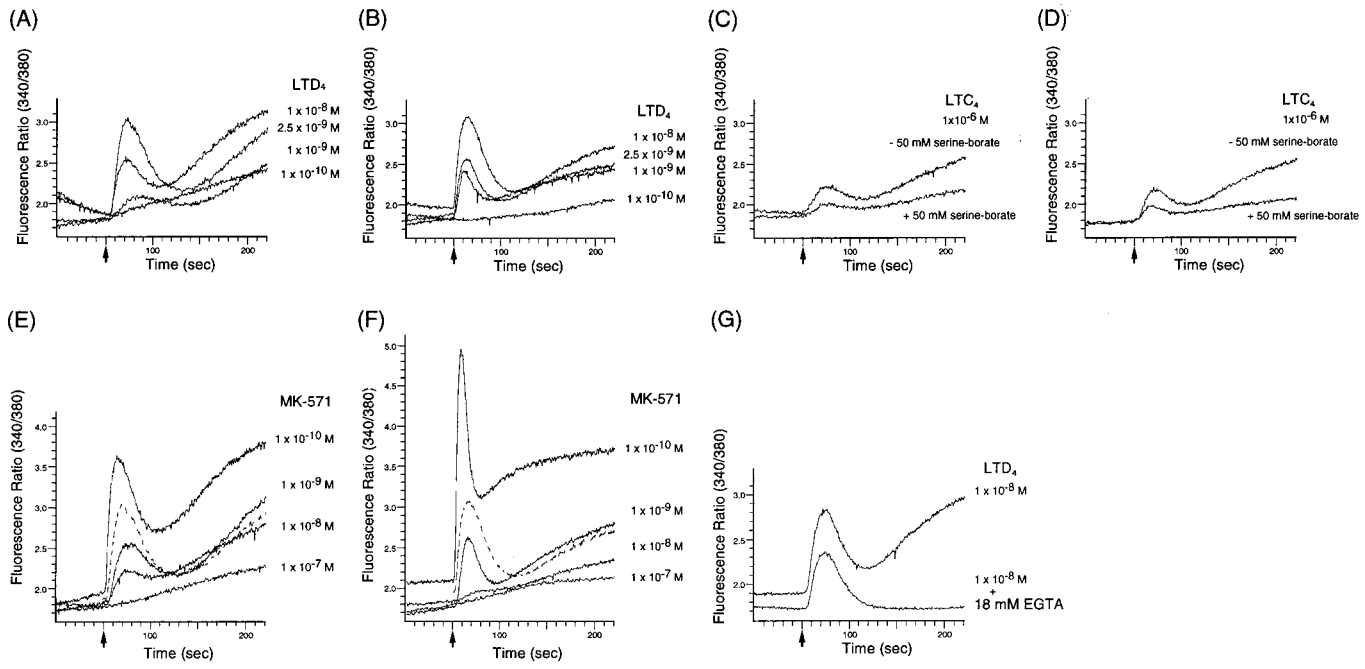

Fig. 3. Calcium mobilization in CHO cells expressing the short (*A*, *C*, *E*, and *G*) and the long (*B*, *D*, and *F*) isoforms of the mouse $CysLT_1$ receptor. The arrows indicate the point of injection of LTs. (*A* and *B*) Dose-dependent responses of the transformants to LTD_4. (*C* and *D*) Responses to 1×10^{-6} M LTC_4 in the presence or absence of 50 mM serine-borate. (*E* and *F*) Responses to 1×10^{-8} M LTD_4 in the presence of various concentration of MK-571; broken lines show the responses in the absence of MK-571 from *A* and *B* of this experiment. (*G*) Response to 1×10^{-8} M LTD_4 in the presence or absence of 18 mM EGTA. Results are representative of at least three independent analyses.

The human $CysLT_1$ receptor is not likely to have the N terminus amino acid extension because there is a stop codon 6 nt upstream of the ATG start codon within the coding exon (Fig. 1*B*) (12). Thus, two isoforms of the mouse $CysLT_1$ receptor were isolated from the mouse lung cDNA library, the short form encoding 339 amino acid residues, which is an equivalent of the human $CysLT_1$ receptor, and the long form encoding 352 amino acid residues. Sequence alignment and comparison of the human and the mouse $CysLT_1$ receptor cDNAs shows that the human contains nucleotide sequences equivalent to the mouse exons I, II, and IV. The isolation of additional cDNAs for the human $CysLT_1$ receptor may uncover the equivalent of the mouse exon III.

Because the 10.5-kb genomic fragment did not contain exon I revealed by the long isoform cDNA, we performed PCR with C57BL/6 mouse genomic DNA as a template and a set of selective primers, designated SD4R-2 for the 5′-CAGGAAGAAGAGTCGAGGTCTATT-3′ oligonucleotide (located in exon I) and ASD4R-2 for the 5′-CTCTATCTGGTACCTCAGCACCTT-3′ oligonucleotide (located in exon II). We sequenced the PCR product and determined the exon I/intron I junction of the mouse $CysLT_1$ receptor gene, which showed that the mouse $CysLT_1$ receptor gene spans approximately 24 kb. The four intron/exon junctions each followed the GT-AG rule (Fig. 1*C*) (30).

Expression of the $CysLT_1$ Receptor mRNA in Mouse Tissues. To determine the tissue distribution of the mouse $CysLT_1$ receptor, we performed Northern blot analysis with the common coding region of the cDNA as a probe. Mouse $CysLT_1$ receptor mRNA was found in the lung and the skin and was hardly detected in the other tissues tested (Fig. 2*A*). The size of the transcripts detected in the lung and skin was approximately 3.5 kb. To further determine the tissue expression of the $CysLT_1$ receptor and to examine whether one of the two mouse $CysLT_1$ receptor isoforms is preferentially expressed in various mouse tissues, we performed RT-PCR on their isolated mRNA with a pair of oligonucleotide primers, SD4R-2 and ASD4R-1, allowing detection of both the long (628-bp product) and the short (470-bp product) isoforms of the mRNA (Fig. 2*B*). A 628-bp product corresponding to the long isoform message was detected intensely in the lung and the skin and weakly in the heart, kidney, stomach, and tongue; this finding was consistent with the Northern blot results. In addition, the lung and skin exhibited a 470-bp product appropriate in size for the short isoform transcript. We confirmed that the PCR products were amplifications of the two isoforms of the mouse $CysLT_1$ receptor by Southern blot analysis with a 580-bp ^{32}P-labeled probe from -410 to $+170$ that was amplified from the long isoform cDNA template by means of a sense primer located downstream of the SD4R-2 primer and an antisense primer located upstream of the ASD4R-1 primer.

The presence of both isoforms of the mouse $CysLT_1$ receptor in lung tissues is consistent with our original isolation of their encoding cDNAs from a mouse lung library. The tissue expression of the mouse $CysLT_1$ receptor mRNA is similar to that in the human with the exception of its lack of detection in the spleen, which was the tissue with highest expression of the human $CysLT_1$ receptor among those examined (11, 12). Furthermore, it is the long isoform of the mouse $CysLT_1$ receptor and not the short isoform, a near counterpart of the human $CysLT_1$ receptor, that is preferentially expressed in the mouse tissues (Fig. 2).

Intracellular Flux of Calcium in the CHO Cells Expressing Distinct Isoforms of the Mouse $CysLT_1$ Receptor. To determine the function of the two isoforms of the mouse $CysLT_1$ receptor, we measured Ca^{2+} flux in Fura-2 AM preloaded CHO cells that stably expressed the long or the short isoform of the mouse $CysLT_1$ receptor. Stimulation of each transformant with LTD_4 elicited a biphasic increase in the fluorescence ratio, reflecting increases in intracellular calcium concentration (Fig. 3 *A* and *B*). The response to LTD_4 was dose dependent at concentrations between 1×10^{-10} and 1×10^{-8} M. Each transformant showed only a slight response to 1×10^{-6} M LTC_4 in the presence of

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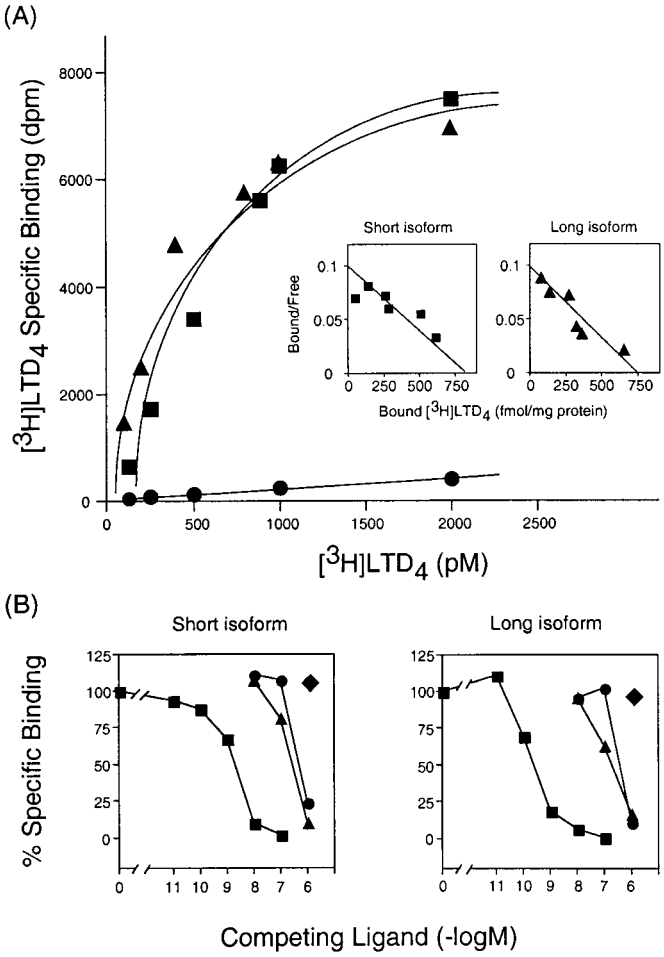

Fig. 4. [^{3}H]LTD$_4$ binding to membrane proteins from CHO cells expressing the mouse CysLT$_1$ receptor. (*A*) Saturation analysis of [^{3}H]LTD$_4$ binding to CHO cell membranes from transformants expressing an isoform of the mouse CysLT$_1$ receptor (■, short isoform; ▲, long isoform; ●, negative control). (*Insets*) Scatchard plot analyses of the short isoform (*Left*) and the long isoform (*Right*) are also shown. (*B*) Competition for [^{3}H]LTD$_4$ binding to CHO cell membranes expressing the short isoform (*Left*) and the long isoform (*Right*) of the mouse CysLT$_1$ receptor (■, LTD$_4$; ●, LTC$_4$; ▲, LTE$_4$; ◆, LTB$_4$). Results are representative of at least three independent analyses.

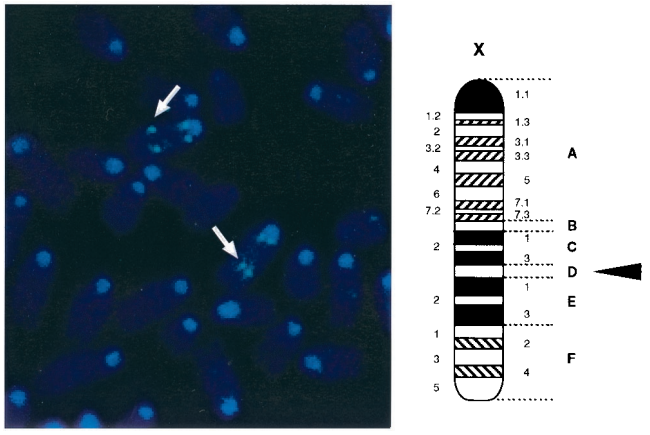

Fig. 5. Chromosomal localization of the mouse CysLT$_1$ receptor. A fluorescence-labeled DNA fragment containing the mouse CysLT$_1$ receptor gene was used to probe stimulated embryo fibroblasts in metaphase. (*Left*) The arrows in the photograph indicate the signal of the CysLT$_1$ receptor gene. The signals observed near the centromere are hybridization with the X chromosome-specific probe. (*Right*) Schematic demonstration of the X chromosome. The area corresponding to band XD containing the CysLT$_1$ receptor alleles is depicted by an arrow.

serine-borate to inhibit conversion to LTD$_4$ (Fig. 3 *C* and *D*). The response to LTE$_4$ was also minimal at 1×10^{-6} M, and there was no agonist activity for LTB$_4$ (data not shown). The response to 1×10^{-8} M LTD$_4$ of each transformant was blocked in a dose-dependent manner by the CysLT$_1$ receptor antagonist MK-571 at concentrations between 1×10^{-9} M and 1×10^{-7} M (Fig. 3 *E* and *F*). In the presence of 1×10^{-10} M MK-571, the response to 1×10^{-8} M LTD$_4$ was enhanced, being particularly prominent in CHO cells expressing the long isoform cDNA. MK-571 inhibited responses to 1×10^{-6} M LTC$_4$ and LTE$_4$. The second phase of calcium increase in response to LTD$_4$ was blocked by the presence of 18 mM EGTA, indicating that the initial rapid calcium flux is caused by the release of intracellular calcium and the second phase reflects the influx of extracellular calcium (Fig. 3*G*). CHO cells transfected but failing to express a CysLT$_1$ receptor transcript by Northern blot analysis did not respond to the LTs tested (data not shown). The EC$_{50}$ of LTD$_4$ for both isoforms of the mouse CysLT$_1$ receptor in CHO cells (≈2 nM) is similar to the EC$_{50}$ for the human CysLT$_1$ receptor in COS-7 cells and HEK-293 cells (in the range of 2.5 to 35 nM). However, the EC$_{50}$ of LTC$_4$ for the mouse CysLT$_1$ receptor is 1,000-fold higher than that of LTD$_4$, whereas the EC$_{50}$ of LTC$_4$ for the human CysLT$_1$ receptor is only 10-fold that of LTD$_4$. Thus, although the LTD$_4$ response and MK-571 inhibition are similar for the mouse and the human CysLT$_1$ receptors, the ligand profile for these transformants is slightly different, with the mouse CysLT$_1$ receptor having little response to LTC$_4$. This characteristic could be either intrinsic to the mouse and the human CysLT$_1$ receptors or merely a consequence of differences in γ-glutamyl transpeptidase activities during the assays.

[^{3}H]LTD$_4$ Binding Assay. We performed [^{3}H]LTD$_4$ binding assays by using membrane protein prepared from CHO cells transfected with either the short or the long isoform cDNA. The membrane preparations showed a similar capacity to bind [^{3}H]LTD$_4$ in proportion to the concentration of the ligand (Fig. 4*A*), and by Scatchard analysis, both showed a single class of binding site for [^{3}H]LTD$_4$ with a K_d of 0.26 nM and a B_{max} of 750 fmol/mg protein (Fig. 4*A Insets*). The membrane from negative control CHO cells showed no specific [^{3}H]LTD$_4$ binding. The binding of [^{3}H]LTD$_4$ in both membrane preparations was inhibited by cold LTD$_4$ in a dose-dependent manner (Fig. 4*B*). LTC$_4$ and LTE$_4$ inhibited the [^{3}H]LTD$_4$ binding at concentrations at least 1,000-fold higher than that for cold LTD$_4$, whereas LTB$_4$ was inactive even at a concentration of 1×10^{-6} M. The binding characteristics and K_d values of both isoforms of the mouse CysLT$_1$ receptor were similar to those of the human CysLT$_1$ receptor (11, 12); but, again, LTC$_4$ was relatively less active for the mouse CysLT$_1$ receptor than for the human CysLT$_1$ receptor.

Chromosomal Localization of the Gene for the Mouse CysLT$_1$ Receptor. With the 10.5-kb genomic fragment containing the mouse CysLT$_1$ receptor used as a probe, specific labeling was observed in the distal region of a large-sized chromosome, which was considered to be the X chromosome on the basis of 4,6-diamidino-2-phenylindole staining. This conjecture was confirmed by cohybridization with a probe specific for the centromeric region of the X chromosome (Fig. 5 *Left*). A total of 62 metaphase cells with specific labeling were analyzed. Measurements of 10 specifically labeled X chromosomes demonstrated that the mouse CysLT$_1$ receptor gene is 59% of the distance from the heterochromatic-euchromatic boundary to the telomere, an

area that corresponds to band XD (Fig. 5 *Right*), which is syntenic to the chromosomal localization of the human $CysLT_1$ receptor gene (11).

Although the human $CysLT_1$ receptor mediates bronchoconstriction of individuals with and without asthma (9), such a response has not been established for normal or antigen-sensitized mice or isolated mouse smooth muscle preparations (20, 21). However, the CysLTs are constrictors of the microvascular smooth muscle, leading to increased permeability and extravasation of blood plasma *in situ* during an inflammatory response in mice (23–25). Furthermore, the CysLTs modulate the lung eosinophil response to aerosol challenge in antigen-sensitized mice (22) and movement of dendritic cells to regional lymph nodes during an immune response (31), as revealed by attenuation of these cellular events with introduction of the $CysLT_1$ receptor antagonist, MK-571. The mouse $CysLT_1$ receptor gene is highly homologous to the human $CysLT_1$ receptor gene in nucleotide sequence but differs somewhat in organization and in the provision of two alternatively spliced transcripts, the long and short isoforms (Figs. 1 and 2). The long isoform has an in-frame start codon 39 nt upstream of the ATG codon of the short isoform, which is most homologous to the human $CysLT_1$ receptor, and thus encodes a protein with a 13-aa extension at the N terminus. Both the mouse and the human $CysLT_1$ receptors, when transfected to supply stable transformants, respond to LTD_4 with an EC_{50} of 2–3 nM and with LTD_4-induced calcium flux that is fully inhibited by MK-571. However, these receptors differ slightly in that the mouse $CysLT_1$ receptor is 1/1,000 less reactive to LTC_4 in the calcium flux assay and in blocking for $[^3H]LTD_4$ binding when compared with LTD_4 (Figs. 3 and 4), whereas this difference for the human $CysLT_1$ receptor is only 1–2 logs (11, 12). Both the mouse and the human $CysLT_1$ receptors reside on a syntenic region of the X chromosome (Fig. 5) (11). Although the genomic organization of the human $CysLT_1$ receptor gene has not been reported, the human cDNA appears to be composed of exons equivalent to the mouse exons I, II, and IV, whereas the mouse long isoform consists of four exons and the short isoform of only exons I and IV. These findings suggest that the transcription of the $CysLT_1$ receptor is differentially regulated in the mouse and the human.

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