Leukotriene B₄ (LTB₄) is a product of eicosanoid metabolism and acts as an extremely potent chemoattractant mediator for inflammation. LTB₄ exerts positive effects on the immigration and activation of leukocytes. These effects suggest an involvement of LTB₄ in several diseases: inflammatory bowel disease, psoriasis, arthritis, and asthma. LTB₄ elicits actions through interaction with one or more cell surface receptors that lead to chemotaxis and inflammation. One leukotriene B₄ receptor has been recently identified (LTB₄-R1). In this report we describe cloning of a cDNA encoding a novel 358-amino acid receptor (LTB₄-R2) that possesses seven membrane-spanning domains and is homologous (42%) and genetically linked to LTB₄-R1. Expression of LTB₄-R2 is broad but highest in liver, intestine, spleen, and kidney. In radioligand binding assays, membranes prepared from COS-7 cells transfected with LTB₄-R2 cDNA displayed high affinity (Kᵦ = 0.17 nM) for [³H]LTB₄. Radioligand competition assays revealed high affinities of the receptor for LTB₄ and LTB₅, and 20-hydroxy-LTB₄, and intermediate affinities for 15(S)-HETE and 12-oxo-ETE. Three LTB₄ receptor antagonists, 14,15-dehydro-LTB₄, LTB₄-3-aminopropylamide, and U-75302, had high affinity for LTB₄-R1 but not for LTB₄-R2. No apparent affinity binding for the receptors was detected for the CysLT1-selective antagonists montelukast and zafirlukast. LTB₄ functionally mobilized intracellular calcium and inhibited forskolin-stimulated cAMP production in 293 cells. The discovery of this new receptor should aid in further understanding of the roles of LTB₄ in pathologies in these tissues and may provide a tool in identification of specific antagonists/agonists for potential therapeutic treatments.

Leukotriene B₄ (LTB₄) is derived as a product of eicosanoid metabolism and is a pro-inflammatory lipid mediator that potently stimulates neutrophil chemotaxis to sites of inflammation (1–3). LTB₄ is involved in the following events: stimulating immigration of leukocytes from the blood stream (4, 5); neutrophil activation leading to degranulation and release of noxious mediators, enzymes, and superoxides (6); inflammatory pain (7); host defense against infection (3); and increased interleukin production (8) and transcription (9). These processes have been implicated in the pathogenesis of a variety of diseases such as inflammatory bowel disease (IBD), psoriasis, arthritis, and asthma (10, 11). Considerable efforts have been devoted in the development of antagonists targeting the cell surface receptors, by screening compounds with radioligand binding assays utilizing membrane preparations from cells such as neutrophils. Potential treatments of various inflammatory conditions with these antagonists have been recently illustrated in human and animal models (11–15).

Extensive studies of LTB₄ and the search for the molecular identity of its receptors have resulted in the recent cloning of a LTB₄ receptor (16) (LTB₄-R1). This protein is a cell surface receptor and belongs to the G-protein-coupled receptor superfamily containing seven membrane-spanning domains. The LTB₄ receptor binds LTB₄ with high affinity, which in turn leads to intracellular signaling and chemotaxis. Among the major tissues tested, the receptor is expressed abundantly only in peripheral leukocytes (16). In this report, we describe the identification of a novel LTB₄ receptor (LTB₄-R2) that shares homology with LTB₄-R1, and the finding that the two receptors are genetically linked. This novel receptor is highly expressed in several peripheral tissues such as liver, spleen, and intestine and binds LTB₄ with high affinity. The ligand-receptor interaction activates the receptor leading to intracellular signal transduction.

EXPERIMENTAL PROCEDURES

Materials—[³H]-labeled LTB₄ (1–200 Ci/mmol) was purchased from PerkinElmer Life Sciences. Human Marathon-ready cDNAs and RACE kit were from CLONTECH. The 293-EBNA cell line was obtained from Invitrogen. Leukotrienes and other ligands were purchased from Sigma Chemicals and Cayman Chemical Co. 14,15-Dehydro-LTB₄, LTB₄-3-aminopropylamide, and U-75302 were purchased from BIORAD Research laboratories, Inc. (Plymouth Meeting, PA). Oligonucleotides were custom-synthesized by Life Technologies, Inc. Their sequences were: oligo347, 5'-cttgccacactgtagcttcatgaca-3'; oligo348A, 5'-gtgacctacttcatcctgtgcaccga-3'; oligo348B, 5'-tgctctacgtcttcaccgctggaga, oligo358, 5'-ggcgcactaatggtgttcggtcgc-3'; oligo417, 5'-gggcgcacctactgactacactcctg-3'; oligo418, 5'-gtgacctacttcatcctgtgcaccga; MM311, 5'-ctgcaagagattgaga-3'; 63U, 5'-tttttgttggaggggagagggaga-3'; 480L, 5'-ggacagccgceccctgacctt-3'; and oligo359, 5'-gggctgctcctgtgactgctgactc-3'. DNA sequencing was performed using the Big Dye Terminators sequencing agents (Applied Biosystems).

Cloning and Sequencing of the New LTB₄ Receptor—The amino acid sequences of known G-protein-coupled receptors were used to conduct a BLAST search against expressed sequence tag data bases. The search identified a 397-base sequence as a putative GPCR fragment.
(HDPYA90R, see Fig. 1A). A phylogenetic analysis (Wisconsin Package, Genetics Computer Group, Madison, WI) suggested that the sequence was related to a leukotriene receptor covering transmembrane domains 3 through 6. Further computational survey of public data bases identified contiguous sequences that resulted in a composite 2451-base sequence of a 1088-base slice, which appeared to be a portion of an open reading frame of a GPCR (Met to TM6, see Fig. 1A). A 3’-RACE was performed to obtain the missing 3’-portion of the putative open reading frame (ORF) by PCR using the Marathon RACE kit for PCR reactions and human liver Marathon-ready cDNA (CLONTECH) as a template. Primary PCR using oligo347 and AP1 (35 cycles of 94 °C for 30 s, 68 °C for 30 s, and 72 °C for 2 min), secondary PCR using primers AP2 and oligo348A (35 cycles of 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 2 min), and tertiary PCR using primers AP2 and oligo345B (35 cycles of 94 °C for 30s, 65 °C for 30 s, and 72 °C for 2 min) resulted in a ~400-base pair (bp) 3’-RACE product (see Fig. 1A). To obtain a full ORF, a 5’-primer (oligo355B) containing the ATG codon in the 888-base sequence and a 3’-primer (oligo359) containing the putative stop codon in the 3’-RACE sequence were generated. A PCR with this pair of primers and human liver cDNA as a template (35 cycles of 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 2 min) yielded a PCR product of ~1.1 kb (SP9630, Fig. 1A).

**Isolation of Genomic Clone—**A genomic clone containing both LTβ2-R2 (SP9300) and LTβ1-R1 receptors was obtained by PCR screening a human placenta cDNA pool (Genome Systems, St Louis, MO) with oligomers 63U and 480L. PCR was performed using PCR SuperMix (Life Technologies, Inc) with a thermal cycling of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s (35 cycles). The size of the intron was determined by PCR using Supermix HighFidelity (Life Technologies) with primers oligo347 and MM311 and the PAC DNA as a template (94 °C for 30 s, 55 °C for 30 s, and 68 °C for 5 min). The resulting PCR product (~4.0 kb) was gel-purified with a Qiaex II gel extraction kit (Qiagen); a genomic clone containing both the ORF of LTB4-R2. The DNA fragment was random primed-labeled with [32P]dCTP, and the blots were hybridized for 14 h in ExpressHyb (CLONTECH) containing ~2 million cpm/ml radiolabeled probe. The following day the blots were washed according to the manufacturer’s protocol and exposed to Kodak Biomax MS film for 6 days at 70 °C. The films were analyzed for relative expression levels using the MCID M4 image analysis system (Imaging Research, Ontario, Canada). For the known receptor LTβ1-R1, a 425-bp fragment corresponding to nucotides 49–474 of the published LTβ1 open reading frame (ORF) of LTβ1-R2. The DNA fragment was random prime-labeled with [32P]dCTP, and the blots were hybridized for 14 h in ExpressHyb (CLONTECH) containing ~2 million cpm/ml radiolabeled probe. The following day the blots were washed according to the manufacturer’s protocol and exposed to Kodak Biomax MS film for 6 days at 70 °C. The films were analyzed for relative expression levels using the MCID M4 image analysis system (Imaging Research, Ontario, Canada). For the known receptor LTβ1-R1, a 425-bp fragment corresponding to nucotides 49–474 of the published LTβ1 open reading frame (ORF) of LTβ1-R2. The DNA fragment was random prime-labeled with [32P]dCTP, and the blots were hybridized for 14 h in ExpressHyb (CLONTECH) containing ~2 million cpm/ml radiolabeled probe. The following day the blots were washed according to the manufacturer’s protocol and exposed to Kodak Biomax MS film for 6 days at 70 °C. The films were analyzed for relative expression levels using the MCID M4 image analysis system (Imaging Research, Ontario, Canada).

**Sequence Analysis—**Sequence analysis of the 1.1-kb PCR product resulting from multiple RACE amplification steps identified a putative ORF of 1077 bp (Fig. 1A), which encodes a protein of 358 amino acids (Fig. 1B). Hydrophobicity analysis of the 358-amino acid sequence suggested that there are seven transmembrane-spanning regions. BLAST analysis with the amino acid sequence against the GenBank® database revealed homology of the amino acid sequence to the human leukocyte LTβ1 receptor (LTβ1-R1, 42%) (16) (Fig. 1B), the human CRTH2 (32%) (17), and the human somatostatin receptor SSTR4 (27%) (18, 19). The amino acid sequence of the receptor is only distantly related (~18% homology) to the recently cloned leukotriene D₄ receptor (20, 21). The high amino acid sequence homology to LTβ1-R1 and the presence in all seven transmembrane domains of conserved amino acid motifs indicated that this was a G-protein-coupled receptor. Thus the novel receptor was tentatively termed as LTβ2-R2.

**Alignment of the cDNA of LTβ2-R2 with the ORF of LTβ1-R1 revealed that the 5’-untranslated region of the ORF of LTβ2-R2. This analysis suggests that portions of both LTβ2 receptors could exist on a single mRNA and the two LTβ2 receptors are in the same chromosomal region. A genomic clone containing coding regions of LTβ2-R2 and LTβ1-R1 was obtained (PAC clone 159K10) by PCR screening of a human PAC library using primers 63U and 480L in the coding region of LTβ2-R2 (Fig. 1A).
Direct sequencing of the PAC clone revealed no intervening sequences in the coding region of either receptor, thus both receptors are encoded by intronless ORFs (Fig. 2A). A second PCR using primers oligo347 and MM311 revealed a single 3.6-kb intron downstream of LTB4-R2 and 5' upstream of LTB4-R1 (Fig. 2A). GenBank entry AL096870 is a genomic sequence from chromosome 14. This fragment contains both the LTB4-R1 and LTB4-R2 genes, as determined by BLAST.

The genomic sequence containing both LTB4-R2 and LTB4-R1 was compared with several mRNA transcripts of LTB4-R2 and LTB4-R1 (Fig. 2, B–D). The 3'-untranslated sequence of LTB4-R2 is identical to the 5'-end of the intron, suggesting that this transcript contains only LTB4-R2 (Fig. 2B). There were three GenBank entries that contained LTB4-R1 mRNAs: D89078, D89079, and U33448. D89079 (16) contains the coding sequences of both LTB4-R2 and LTB4-R1 but does not have the 3.6-kb intron (Fig. 2C). U33448 (22) contains only the coding region of LTB4-R1, and the 5'-untranslated sequence is identical to the 3'-end of the 3.6-kb intron sequence (Fig. 2D). D89078 also contains only the coding region of LTB4-R1; however, the 5'-untranslated region is identical to the middle portion of the 3.6-kb intron (Fig. 2E).

Dot blot and Northern blot analyses were performed to determine the expression of LTB4-R2 mRNA in human tissues. A dot blot containing mRNAs from 56 human tissues (CLONTECH) was hybridized to a 440-bp fragment derived from the 5'-UTR of LTB4-R2 cDNA. The highest expression of LTB4-R2 was detected in liver followed by small intestine, spleen, and fetal liver (20–40% of that of liver; Fig. 3A). Adrenal gland and pituitary had expression levels between 10 and 20% of those in the liver. All of the other 49 tissues expressed LTB4-R2 at 10% of or less than that of the liver level (Fig. 3A). Using the same fragment as probe to hybridize a Northern blot, a mRNA of approximately 1.6 kb with high abundance was seen in the liver and a weak band in kidney (Fig. 3B). No expression was detected in heart, brain, placenta, skeletal muscle, and pancreas. Employing a quantitative PCR method (Taqman, PE Biosystems) with 27 cDNA preparations generated primarily from human fetal and diseased tissues as templates, the highest expression of LTB4-R2 was again detected in fetal small intestine, Crohn's colon, fetal liver, and fetal lung (not shown). As is the case for the novel LTB4 receptor, the LTB4-R1 mRNA appears to be widely distributed in human tissues based on the results of the dot blot. LTB4-R1 is most abundant in immune-related tissues, including spleen, peripheral leukocytes, and bone marrow. Although there is also low expression of the LTB4-R1 mRNA in liver, it is not as prominent as that for LTB4-R2. The Dot blot data for LTB4-R1 are consistent with the Northern blot analysis shown by Yokomizo et al. (16)
in which high expression was seen in peripheral leukocytes and low or no mRNA was detected in other tissues.

Radioligand binding assays were performed to directly test the ability of LTB₄-R2 to bind LTB₄. The ORF of LTB₄-R2 was cloned in expression vector pCR3.1 to form construct pCR3.1-LTB₄-R2. COS-7 cells were transfected with the construct, and membranes were prepared for a [³H]LTB₄ binding assay. As shown in Fig. 4A, specific binding was observed with the membranes prepared from cells transfected with pCR3.1-LTB₄-R2; in contrast, no specific binding was seen with membranes prepared from cells transfected with vector alone. Because serum used in the cell cultures may carry low concentrations of LTB₄ (23), radioligand binding assays were performed in parallel with membranes prepared from cells grown for the last 24 h in either serum-free (Opti-MEM) or medium containing 5% (v/v) FCS. No difference in the abilities of the two membrane preparations to bind [³H]LTB₄ were found (Fig. 4A), indicating that the serum used in the experiments did not affect ligand-receptor interaction through potential effects of either desensitization or receptor down-regulation. Saturation radioligand binding assays employing membranes from cells cultured in serum yielded a $K_a$ of 0.17 ± 0.07 nM and $B_{max}$ of 70 ± 8 fmol/mg of membrane protein ($n = 3$) (Fig. 4B). Similar $K_a$ and $B_{max}$ values were obtained when membranes were prepared from cells cultured in serum-free medium: $K_a = 0.21 ± 0.06$ nM and $B_{max} = 64 ± 7$ fmol/mg of protein (not shown).

The pharmacological profiles of LTB₄-R2 and LTB₄-R1 were compared in radioligand competition assays using [³H]LTB₄ as the radioligand and a number of unlabeled leukotrienes, leukotriene analogs, leukotriene receptor antagonists, and 5-lipoxygenase products as competitors (Table I). LTB₄, LTB₅, and an LTB₄ metabolite, 20-hydroxy-LTB₄, have high affinities for both LTB₄-R2 ($K_i ≤ 41$ nM and relative affinity = 18) and LTB₄-R1 ($K_i ≤ 3.7$ nM and relative affinity = 5.3) receptors (Table I and Fig. 5). A 5-lipoxygenase product, 15(S)-HETE, and an arachidonic acid derivative, 12-oxo-ETE, displayed moderate affinities for LTB₄-R2 (relative affinity = 67–73) but had low binding affinities for LTB₄-R1 ($K_i > 1000$ nM) (Table I and Fig. 5). The affinities of the three LTB₄ receptor antagonists (14,15-dehydro-LTB₄ (24), LTB₄-3-aminopropylamide (25, 26), and U-75302 (27)) were relatively lower for LTB₄-R2 ($K_i = 473–5434$ nM) than for LTB₄-R1 ($K_i = 5.1–27$ nM) (Table I). Another LTB₄ metabolite, 20-carboxy-LTB₄, bound LTB₄-R1 with high affinity ($K_i = 20$ nM) but bound LTB₄-R2 with much lower affinity ($K_i > 1000$ nM) (Table I).

Other test compounds displayed generally low affinities for the LTB₄-R2 and LTB₄-R1 receptors in the competition assay (Table I). These compounds include several 5-lipoxygenase products such as 5(S)-HETE, 20-HETE, 8(S)-HETE, and 5(S)-HETE (relative affinities > 435, Table I), two arachidonic acid derivatives (5-oxo-ETE and 5,6-dehydroarachidonic acid, relative affinities > 435), and lipoxin-A₄ with affinities of $1400$ nM ($K_i$) for the two receptors (Table I). 6-trans-LTB₄, a trans stereoisomer of LTB₄, also bound the receptors with relatively low affinity (Table I). Leukotrienes in the C, D, E, and F families and methyl ester of the leukotriene As, displayed weak or no affinities for LTB₄-R2, as well as LTB₄-R1 (Table I). Two CysLT₁-antagonists, montelukast (Singulair) and zafirlukast (Accolate) that have high affinities for the LTA₄ receptor (20, 21), had affinities at least 4000 times lower than that of LTB₄ for LTB₄-R2 and LTB₄-R1 (Fig. 5, Table I).

The ability of LTB₄-R2 receptor to mediate intracellular signal transduction was examined by measurements of fluorescence of Fluo-3, AM as intracellular Ca²⁺ flux and by assays of forskolin-induced cAMP production. Interaction of LTB₄-R2 expressed in 293-EBNA cells with LTB₄ activated cellular Ca²⁺ release (Fig. 6A), suggesting a functional coupling of LTB₄-R2 with intracellular G-proteins. In contrast, cells that were mock-transfected with vector alone did not respond to incubation with LTB₄ (Fig. 6A). The ability of the receptor to mediate inhibition of forskolin-stimulated intracellular cAMP was tested in 293 cells expressing LTB₄-R2. LTB₄ caused a dose-dependent inhibition of the cAMP production (Fig. 6B). Non-linear regression analysis of the data yielded a maximum inhibition of about 60% and EC₅₀ of 58 ± 20 nM ($n = 2$). Incubation of the cells with 100 ng/ml PTX reversed most of the inhibitory
activity, indicating that this is a PTX-sensitive pathway (Fig. 6B).

DISCUSSION

A thorough understanding of the roles of LTB₄ requires identification and characterization of all the LTB₄ receptors. We have cloned and characterized a novel LTB₄ receptor that is different from and genetically linked to the previously cloned LTB₄ receptor (LTB₄-R1). The two receptors vary in primary structures displaying only 42% homology and in tissue expression patterns, but both are able to bind LTB₄ with high affinity and are functional in stimulating intracellular signaling. In addition, the two receptors are genetically linked at one locus of the genome.

The cloned LTB₄-R2 binds LTB₄ with highest affinity among the 31 ligands tested. The binding affinity is comparable to that of LTB₄ for the previously published LTB₄-R1 receptor (Table I), indicating that the receptor is a LTB₄ receptor subtype. Consistent with this conclusion are the observations that LTB₄-R2 shares a high homology to LTB₄-R1 and that the two receptors are genetically co-localized (Figs. 1 and 2). The ligand affinity of LTB₄-R2 extends from LTB₄ only to LTB₅ and 20-hydroxy-LTB₄, whose structures are closely related to LTB₄, suggesting a specific ligand recognition (Table I). High affinity binding of 20-hydroxy-LTB₄ to human PMNs has been observed previously (28); it is more soluble than and expresses functional activities similarly to LTB₄, suggesting a more important role of 20-hydroxy-LTB₄ in inflammation than LTB₄ (28). Except the moderate affinity of 12-oxo-ETE and 15(S)-HETE for LTB₄-R2, all the other ligands tested, including analogs of LTB₄ or 5-lipoxygenase products, do not bind the receptor with high affinity (Table I). We note that clinically used LTD₄ receptor antagonists montelukast and zafirlukast have no affinity for the receptor (Fig. 5), consistent with the finding that the two LTB₄ receptors share low homology with...
The LTD₄ receptor. Although LTB₄ and LTD₄ are derived from a common metabolic pathway, their receptors are nevertheless phylogenetically distant, suggesting the use of a common pathway in regulations of diverse physiological functions.

The two receptors possess different pharmacological profiles. Like LTB₄-R₂, LTB₄-R₁ binds LTB₄, LTB₅, and 20-hydroxy-LTB₄ with high affinity (Table I). However, several compounds, including 14,15-dehydro-LTB₄, LTB₄-3-aminopropylamide, U-75302, and 20-carboxy-LTB₄, which do not bind LTB₄-R₂ tightly, bind LTB₄-R₁ with high affinity (Table I). In contrast, 12-oxo-ETE and 15(S)-HETE showed preferential binding to LTB₄-R₂ (Table I). The trans stereoisomer of LTB₄ (6-trans-LTB₄) displayed low affinity for either LTB₄-R₁ or LTB₄-R₂ (Table I), suggesting trans stereospecificity at C-6 of LTB₄ for these receptors (29).

Activation of the LTB₄-R₂ receptor leads to variable signaling events, including the mobilization of Ca²⁺ and modulation of intracellular cAMP levels. Activation of the receptor leads to the interaction of the receptor with two classes of G-protein: the Gq class, which mediates cellular Ca²⁺ release, or the Gi class, which mediates inhibition of forskolin-stimulated cAMP production in a PTX-sensitive manner. The activation of multiple signal transduction pathways suggests different roles for LTB₄ through the interaction with LTB₄-R₂, depending on the availability of G-protein reserves in different cell types.

LTB₄ activities may be regulated by differential expression of the receptors in different tissues. The new LTB₄ receptor is highly expressed in the liver, intestine, and spleen, and to some degree, in the kidney (Fig. 3), suggesting that this receptor regulates LTB₄-mediated functions in these tissues. This expression pattern is in contrast to that of LTB₄-R₁, which is expressed only in the peripheral leukocytes (16). The differential expression patterns (Ref. 16, Fig. 3) suggest the functions of the two receptors are tissue-specific and may be involved in different inflammatory processes associated with immune or hepatointestinal systems. The characteristics of LTB₄-R₂ as an active cell surface receptor that binds radiolabeled LTB₄ saturably (Fig. 4) and stimulates intracellular signaling (Fig. 6) support LTB₄ acting as stimulant for cellular functions other than merely being passively metabolized by liver cells.

Relatively high expression of LTB₄-R₂ is consistent with existing evidence implicating functions of LTB₄ in these tissues. Hepatic macrophages secreting LTB₄ attract neutrophils to the liver in rats with septic liver injury (30). In a hepatic ischemia-reperfusion injury model, rat liver LTB₄ levels were increased to levels 50-fold those in control liver, accompanied with increase of plasma alanine aminotransferase activities and polymorphonuclear leukocyte accumulation in the liver (31). Only the concentration of LTB₄, not LTC₄ and LTE₄, in plasma and stimulated peripheral blood leukocyte supernatants.
**Pharmacological profiles of the LTB₄-R2 and LTB₄-R1 receptors determined with radioligand competition assays**

\[ [3H]LTB_4, at 0.25 nM, was displaced by indicated leukotrienes or compounds from membranes prepared from COS-7 cells transfected with either pCR3.1-LTB₄-R1 or pCR3.1-LTB₄-R2. Kᵈ values are calculated for individual compound by using \( Kᵈ = EC_{50}/nM \times [3H]LTB₄/Kᵈ \) (42), where \([3H]LTB₄\) is the concentration of the radioligand used in the assay, \( Kᵈ\) is the affinity of the radioligand for the receptor (0.2 nM), and \( EC_{50}\) is determined by non-linear regression analysis of the binding data. Results are represented as mean ± S.E. from two to four independent experiments performed in duplicate. \( Kᵈ/Kᵈ(LTB₄)\) denotes relative affinity of ligands in respect to that of LTB₄. The cDNA of LTB₄-R1 was generated in a PCR with primers oligo417 and oligo418 with human spleen cDNA as a template. The thermal cycling profile was 93 °C for 30 s, 72 °C for 90 s (35 cycles). The cDNA was then cloned in expression vector pCR3.1.

| Ligand                  | LTB₄-R2 | LTB₄-R1 |
|-------------------------|---------|---------|
|                         | \( Kᵈ \) | \( Kᵈ/Kᵈ (LTB₄) \) | \( Kᵈ \) | \( Kᵈ/Kᵈ (LTB₄) \) |
| [3H]LTB₄                | 2.3 ± 1.1 | 1       | 0.7 ± 0.4 | 1       |
| LTB₂                   | 9.4 ± 4.8 | 4       | 3.7 ± 3.0 | 5.3     |
| 20-Hydroxy-LTB₄         | 41 ± 12  | 18      | 0.54 ± 21 | 0.8     |
| 15(S)-HETE             | 173 ± 80 | 73      | >18,000   | >25,714  |
| 12-Oxo-ETE             | 155 ± 74 | 67      | >1,000    | >1,429   |
| 14,15-Dehydro-LTB₄     | 473 ± 201| 205     | 27 ± 5    | 38       |
| LTB₃-3-aminopropylamide| 1,227 ± 680| 533       | 5.1 ± 0.5 | 7.3     |
| U-75302                | 5,434 ± 1,320| 2,362     | 25 ± 4    | 36       |
| 20-Carboxy-LTB₄        | >1,000   | >435    | 20 ± 2    | 29       |
| 6-trans-LTB₄ (trans stereoisomer of LTB₄)| >1,000 | >435 | 336 ± 15 | 480     |
| 5-Oxo-ETE              | >1,000   | >435    | >1,000    | >1,429   |
| 5(S)-HETE              | >3,000   | >1,304  | >20,000   | >28,571  |
| (±)-5-HETE             | >1,000   | >435    | >1,000    | >1,429   |
| 20-ETE                 | >1,000   | >435    | >10,000   | >14,285  |
| 8(R)-HETE              | >1,000   | >435    | >30,000   | >42,857  |
| 5,6-Dehydroarachidonic acid | >1,000 | >435 | >1,000    | >1,429   |
| Lipoxin-A₃             | 1,400 ± 110| 699         | 1,440 ± 190| 2,057    |
| Montelukast            | >10,000  | >4,350  | >10,000   | >14,285  |
| Zafirlukast            | >10,000  | >4,350  | >7,124    | >10,177  |
| LTC₃                   | >4,000   | >1,739  | >14,000   | >20,000  |
| LTC₄                   | >9,000   | >3,913  | >1,900    | >4,714   |
| LTC₅                   | >9,000   | >3,913  | >3,000    | >42,587  |
| LTC₆                   | >1,000   | >435    | >30,000   | >42,587  |
| LT₂D₆                 | >10,000  | >4,350  | >4,000    | >5,714   |
| LT₂D₅                 | NA       | >4,350  | >4,000    | >5,714   |
| LTE₄                   | >4,000   | >1,739  | >30,000   | >42,587  |
| LTE₅                   | >6,000   | >2,608  | >2,370    | >3,387   |
| LTE₆                   | >6,000   | >2,608  | >5,700    | >8,142   |
| LTA₃-ME                | >30,000  | >13,040 | >5,000    | >7,142   |
| LTA₄-ME                | >10,000  | >4,350  | >30,000   | >42,587  |
| LTA₅-ME                | NA       | >4,350  | >30,000   | >42,587  |

Higher in rats fed corn oil and ethanol (alcoholic liver) than in animals fed saturated fat and ethanol (no liver injury) (34). Finally, enhanced production of LTB₄ by peripheral blood mononuclear cells in patients was found with fulminant hepatitis (35).

In spleen, intraperitoneal LTB₄ increased the survival rate of mice infected with methicillin-resistant *Staphylococcus aureus* (36). Guinea pig spleen membrane preparations have high affinity for LTB₄ and contain moderate number of LTB₄ binders at 37; this receptor has high affinity for LTB₄ and 20-hydroxy-LTB₄ but low affinity for 20-carboxy-LTB₄ (37), a profile similar to LTB₄-R2 (Table 1). In a rat renal ischemia-reperfusion injury model, Chinese hamster ovary cells expressing a LTB₄ receptor accumulate along with neutrophils in the postischemic kidney; use of LTB₄ antagonists led to the marked decrease in accumulation of Chinese hamster ovary cells and neutrophils (38). Spontaneously hypercholesterolemic rats, characterized by glomerular infiltration of macrophages, fed a normal diet developed end-stage renal failure in 26 weeks, while those fed a diet supplemented with a LTB₄ antagonist showed normal renal function (39). In the intestinal system, high LTB₄ levels in colonic mucosa in patients with IBD were detected (10), more than 10-fold chemotactic activity was found in homogenates of IBD mucosa than in those of normal colonic mucosa, and only lipid extract fraction co-eluted with LTB₄ was chemotactically active (40). Furthermore, the response to IBD mucosa was inhibited by anti-LTB₄ antisera, suggesting that LTB₄ is an important stimulus to neutrophil chemotaxis in the disease (40). Given that LTB₄-R1 is not found in either small...
The close genetic linkage between the two LTB4 receptors (Fig. 2A) is unique to other GPCR families and may play a role in regulating receptor activation. The genomic organization illustrated in Fig. 2A was confirmed by a more recent GenBank entry of a human genomic fragment, AL096870, which contains both LTB4 receptor genes. Because the fragment is located on chromosome 14, we mapped the LTB4-R1 and LTB4-R2 receptors to this chromosome. Although we have observed various transcripts that may contain single receptor of LTB4-R1 or LTB4-R2, or may contain coding regions of both LTB4-R1 and LTB4-R2, the molecular mechanism that regulates the formation of those transcripts from the two genes and the regulatory roles of the transcripts in specific physiological functions are still not clear.

Identification of multiple LTB4 receptors with distinct primary structures, differential pharmacological profiles, and expression patterns points to the potential of differential regulation of LTB4 effects on a variety of inflammatory diseases. Delineation of pleiotropic LTB4 receptor activation is essential for developments of disease- and receptor subtype-specific antagonists/agonists in various therapeutic areas. It will be necessary to define the specificity of the previously reported LTB4 antagonists (13–15, 41) toward the two LTB4 receptor subtypes, should these compounds be successfully developed and safely used in clinic settings. The discovery of the hepatointestinal receptor and illustration of the cellular mechanisms mediated by this receptor should aid in design of further studies to understand the roles of LTB4 in functions of the tissues and identification of receptor subtype-specific antagonists and agonists.

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