The mouse leukotriene B4 receptor (m-BLTR) gene was cloned. Membrane fractions of human embryonic kidney 293 cells stably expressing m-BLTR demonstrated a high affinity and specific binding for leukotriene B4 (LTB4, \( K_d \approx 0.24 \pm 0.03 \text{nM} \)). In competition binding experiments, LTB4 was the most potent competitor \((K_i = 0.23 \pm 0.05 \text{nM})\) followed by 20-hydroxy-LTB4 \((K_i = 1.1 \pm 0.2 \text{nM})\) and by 6,trans-12-epi-LTB4 and LTB4 \((K_i > 1 \mu \text{M})\). In stably transfected Chinese hamster ovary cells, LTB4 inhibited forskolin-activated cAMP production and induced an increase of intracellular calcium, suggesting that this receptor is coupled to \(G_{\alpha_1}\) and \(G_{\beta_\gamma}\) proteins.

In *Xenopus laevis* melanophores transiently expressing m-BLTR, LTB4 induced the aggregation of pigment granules, confirming the inhibition of cAMP production induced by LTB4. BLT receptors share significant sequence homology with chemokine receptors (CCR5 and CXCR4) that act as human immunodeficiency virus (HIV) coreceptors. However, among the 16 HIV/SIV strains tested, the human BLT receptor did not act as a coreceptor for virus entry into CD4-expressing cells based on infection and cell-cell fusion assays. In 5-lipoxygenase-deficient mice, the absence of leukotriene B4 biosynthesis did not detectably alter m-BLTR receptor binding in membranes obtained from glycerol-elicited neutrophils. Isolation of the m-BLTR gene will form the basis of future experiments to elucidate the selective role of LTB4 as opposed to cysteinyl-leukotrienes, in murine models of inflammation.

Leukotriene B4 (LTB4)\(^1\) and the cysteinyl-leukotrienes (LTC4, LTD4, and LTE4), derived from arachidonic acid metabolism, are synthesized sequentially by 5-lipoxygenase and then by either LTA4 hydrolase or LTC4 synthase, respectively. The biological actions of the cysteinyl-leukotrienes are mediated through at least two G protein-coupled receptors referred to as CysLT1 and CysLT2 whose molecular identities remain uncharacterized. LTB4 mediates its effects through a membrane G-protein-coupled receptor termed BLTR (3). Additionally, LTB4 was shown to bind to the intracellular transcription factor peroxisome proliferator-activated receptor (4). This facet of binding has been proposed to be part of a negative feedback mechanism to limit the inflammatory actions of LTB4. However, the binding to peroxisome proliferator-activated receptor has been questioned in recent experiments (5).

LTB4, a dihydroxy fatty acid, is one of the most potent known chemoattractant mediators, acting mainly on neutrophils but also on related myeloid cells, mast cells, and endothelial cells (6, 7). LTB4 induces chemotaxis, chemokinesis, and aggregation, causing the migration of neutrophils to sites of inflammation where the cells degranulate, resulting in the release of lysosomal enzymes in addition to other antibacterial and antimicrobial agents (8). LTB4 also promotes the adherence of neutrophils to vascular endothelial cells and their transmigration, which amplifies the inflammatory response.

LTB4 has been implicated in the pathophysiology of various diseases like arthritis, inflammatory bowel disease, and psoriasis. The exact role of LTB4 in the etiology of these disorders has been debated vigorously. Inhibitors of 5-lipoxygenase and the 5-lipoxygenase-activating protein have been used efficiently in models of ulcerative colitis (9, 10), endotoxic shock (11), and induced asthma (12–14). The development of specific and highly potent BLTR antagonists has lagged behind cysteinyl receptor antagonists, which are currently available in the clinic for treatment of asthmatic inflammatory symptoms. One BLTR antagonist has shown encouraging results in a murine model of collagen-induced arthritis (15).

There has been considerable controversy about the molecular identification of the BLTR. In 1996 two independent research groups (16, 17) cloned identical orphan receptor genes believed to encode members of the chemotactic factor subfamily of G protein-coupled seven transmembrane receptors. Initially, one group indicated that the receptor was unable to bind LTB4 (16) but later retracted this finding to indicate specific binding to embryonic kidney; CHO, Chinese hamster ovary; HEK-m-BLTR, stable transfected HEK cells with pCR3.1-m-BLTR; CHO-m-BLTR, stable transfected CHO cells with pCR3.1-m-BLTR; SIV, simian immunodeficiency virus.
(18). A third group cloned the identical receptor sequence, which was classified as a purinergic P2Y receptor on the basis of its affinity for binding ATP (19). However, Yokomizo et al. (3) challenged this identification and provided convincing proof that the human BLTR (h-BLTR) had been cloned.

Intense interest in the role of chemokine receptors for facilitation of HIV entry into CD4-positive cells is evident from recent surveys of the literature (20, 21). The h-BLTR is structurally related to chemokine receptors (e.g., CCR5) and is expressed in various immune cells. Recent evidence has suggested that the h-BLTR may function as a coreceptor for entry of primary HIV-1 isolates into CD4-positive cells (22). If true, this finding would add a significant new dimension to the interplay of leukotrienes, inflammation, and AIDS pathogenesis.

We report here the cloning and characterization of the m-BLTR, the signaling pathways for this G protein-coupled receptor, and a detailed analysis as to whether the h-BLTR can function as an HIV coreceptor.

**EXPERIMENTAL PROCEDURES**

**Materials**—[α-32P]dCTP was purchased from NEN Life Science Products and [3H]LTB4 from Amersham Pharmacia Biotech. The mouse strain 129 Sv genomic library in Lambda Fix II and the cloning vector pBluescript II KS were from Stratagene (La Jolla, CA), and the mammalian expression vector pCR3.1 Uni was from Invitrogen (Carlsbad, CA). LTb4 20-hydroxy-LTB4, 6-trans-12-epi-LTB4, and LTB4 were purchased from Cayman Chemical Co. (Ann Arbor, MI). Dulbecco’s modified Eagle’s medium, Ham’s F-12, Opti-MEM, L-15, conditioned frog medium, phosphate-buffered saline, fetal bovine serum, and LipofectAMINE were from Life Technologies, Inc., and restriction enzymes were from New England Biolabs (Beverly, MA). Ampli-Taq DNA polymerase was obtained from Perkin-Elmer.

**Screening of Genomic Library**—A 1.1-kb fragment (NheI-PstI) from the cDNA, described in Ref. 19 and labeled with [α-32P]dCTP, was used as probe to screen the genomic mouse library by standard procedures. Putative positive clones were taken through two additional rounds of screening until plaque-purified. Phage DNA was purified and subjected to restriction enzyme digestion and Southern blot analysis.

**Plasmid Constructs**—A 2.2-kb XbaI fragment that hybridized to the probe was gel extracted and inserted into the XbaI site of pBluescript II KS. The insert was sequenced entirely on both strands using automated sequencing (Applied Biosystems Big Dye Terminator, Ready Reaction Kit Reagents; ABI 373 sequencer) at the Department of Genetics, University of Pennsylvania.

**Open reading frame** was amplified by PCR using primers CDF367 (5’-GCCATGCTGCAAAACTACTC-3’) and CDF370 (5’-AGTCTACTGCAGACTAGG-3’). The sequence of the amplified plasmid was confirmed and cloned into the vector pCR3.1 Uni (Stratagene) under control of the T7 promoter. The h-BLTR, previously characterized as the P2Y7 receptor (19), was subcloned from pBluescript II KS into pCR3.1 Uni digestion and Southern blot analysis.

**Experiments**—Cultured Nestor-m-BLTR and CHO-m-BLTR cells were harvested and washed with HEPES-buffered saline. Cells were then loaded with the fluorescent dye FURA-2/AM (Molecular Probes, Eugene, OR) at 10 μM, washed and resuspended in HEPES-buffered saline containing: 142 mM NaCl, 2.4 mM KCl, 1.2 mM KH2PO4, 1.3 mM CaCl2, 10 mM HEPES, pH 7.4, and 250 μM sulfinpyrazone, the latter being added in order to reduce excretion of the dye (23). Measurements of change in Ca2+ levels in stirred cell suspensions were made using a Perkin-Elmer model LS50B luminescence spectrometer and were expressed as ratios of fluorescence emitted at 510 nm in response to excitation at 340 and 380 nm (data sampling interval, 0.5 s). Calcium concentrations were calculated from these ratios after determining the maximum and minimum ratios of fluorescence in the presence and absence of 2.5 μM LTB4. All samples, in duplicate, were incubated at room temperature for 1 h. The total and nonspecific binding were determined as the amount of [3H]LTB4 bound to the membrane fractions in the presence or absence of 2.5 μM LTB4. Bound and free radioligands were separated by filtration through Whatman GFC filters presoaked with 0.1% bovine serum albumin in 10 mM HEPES.

**Intracellular Calcium Measurements**—Confluent CHO-m-BLTR cells were harvested and washed with HEPES-buffered saline. Calcium buffers were then loaded with the fluorescent dye FURA-2/AM (Molecular Probes, Eugene, OR) at 10 μM, washed and resuspended in HEPES-buffered saline containing: 142 mM NaCl, 2.4 mM KCl, 1.2 mM KH2PO4, 1.3 mM CaCl2, 10 mM HEPES, pH 7.4, and 250 μM sulfinpyrazone, the latter being added in order to reduce excretion of the dye (23). Measurements of change in Ca2+ levels in stirred cell suspensions were made using a Perkin-Elmer model LS50B luminescence spectrometer and were expressed as ratios of fluorescence emitted at 510 nm in response to excitation at 340 and 380 nm (data sampling interval, 0.5 s). Calcium concentrations were calculated from these ratios after determining the maximum and minimum ratios of fluorescence in the presence and absence of saturating levels of Ca2+, respectively, according to the ratiometric method described previously (24).

**Functional Bioassay**—Xenopus laevis melanophores were maintained in culture and used as described previously (25–28). Briefly, transient expression of pCR3.1-m-BLTR in melanophores was achieved by electroporation. Melanophores were plated (15,000/well) in 96-well tissue culture plates and cultured for 2 days. Before the addition of agonist, cells were washed, incubated with 0.7 × L-15, 0.1% bovine serum albumin as described (25), and then exposed to room light for 1 h. This exposure causes the cells to disperse their pigment granules and darken. The plates were incubated for 1 h in room light and base-line absorbance (A0) read at 620 nm using a Molecular Devices Spectrophotometer. The absorbance was measured immediately in the microtiter wells in 20-μl aliquots at 10 × their final concentration. Dose-response data were obtained 1 h later (A1). Data were plotted with y = 1 − (A1/A0). Data are presented as mean ± S.E.

**cAMP Assay**—CHO-m-BLTR were plated in 12-well plates at a density of 200,000 cells/well. 2 days later, the cultured medium was replaced with 0.5 ml of culture medium with 25 μM forskolin. After 15 min, different concentrations of LTB4, were added. The medium was removed 10 min after the addition of agonist, and the cAMP produced was extracted by adding 0.5 ml of ethanol to each well. The supernatant was evaporated to dryness, and the pellet was dissolved in Tris (0.05 M) EDTA (4 mM) buffer, pH 7.5. The cAMP content was measured using a [3H]cAMP radioimmunoassay kit from Amersham Pharmacia Biotech

**BLT Receptor Binding Assays to Glycogen-elicited Neutrophil Membranes**—5-Lipoxigenase-deficient mice (29) and wild-type controls (five mice each) from our colony were injected intraperitoneally with glycogen and the neutrophils harvested 5 h later as described previously (29). Binding was carried out on membranes as mentioned above.

**Cell-Cell Fusion and Virus Infection Assays**—For cell-cell fusion assays, CHO-CD4 and CHO-transfected cells were transfected with plasmids expressing CD4, the desired coreceptor, and luciferase under control of the T7 promoter. The h-BLTR, previously characterized as the P2Y2, receptor (gift from S. Kunapuli; Ref. 19), was used for these studies (see “Results”). The next day, Q76 effector cells expressing the desired Env protein by transfection and T7 polymerase as a consequence of infection by recombinant vaccinia viruses were added. In this assay, cell-cell
fusion results in cytoplasmic mixing and luciferase production, which can be easily quantified. Additional details can be found in previous papers (30, 31). For infection assays, we used luciferase reporter viruses. Human 293T cells were transfected with plasmids expressing the desired Env (in a pcDNA3 or psv7d background) and with the NL4–3 luciferase virus backbone (pNL-Luc-E'F') (32, 33). Virus was harvested from the media the next day and used to infect feline CCCS cells (for HXBch) or 293T cells (for all other Evs) expressing the indicated CD4/coreceptor combinations. Infection was quantified by measuring luciferase activity 3-days postinfection.

**Fig. 1.** [3H]LTB₄ binding to membrane fractions of HEK 293 cells transiently or stably (HEK-m-BLTR) transfected with pCR3.1-m-BLTR. Panel A, total binding of 0.2 nM [3H]LTB₄ to membrane fractions (100 µg) of nontransfected cells (Ct) or transiently transfected HEK 293 cells. Total binding (black column) and nonspecific binding (gray column) are shown (mean ± S.E., n = 4). Cells transfected with a control vector (pCR3.1 Uni) have the same total and nonspecific binding as membrane fractions from parental cells (not shown). Panel B, Scatchard analysis and saturation isotherms of [3H]LTB₄ binding to membrane fractions of HEK-m-BLTR cells stably transfected with pCR3.1-m-BLTR. Data from a representative experiment of four giving similar results are shown. The linear Scatchard plot gives a correlation coefficient of 0.99. In this particular experiment the Bₘₐₓ and Kᵣ values were 474 fmol/mg of protein and 0.19 nm, respectively. The specific (●), nonspecific (□), and total binding (○) isotherms are drawn (inset). Panel C, displacement curves of specific [3H]LTB₄ binding using several compounds with structures similar to that of LTB₄. Inhibition of 0.1 nM [3H]LTB₄ binding to the membrane fractions of HEK-m-BLTR cells, by LTB₄ (●), 20-hydroxy-LTB₄ (○), 6-trans-12-epi LTB₄ (△), and LTD₄ (□) is shown. Each point reflects the mean ± S.E. of four experiments.

**LTB₄ Receptors**

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RESULTS

Isolation of m-BLT Receptor Gene—To clarify the confusion surrounding the molecular identity of the h-BLTR and to advance the study of the role of LTB₄ in murine inflammation models, we sought to clone and characterize the m-BLTR. A mouse genomic library was screened using a 1.1-kb fragment from the cDNA identified previously as encoding the purinergic P2Y₇ receptor (19). Among the positive clones that hybridized to the probe, one genomic clone with a 2.2-kb XbaI fragment was found to display an open reading frame of 1,056 kb, encoding a 351-amino acid receptor with seven putative transmembrane domains, two potential glycosylation sites, and several phosphorylation sites (GenBank accession number AF077673).

This open reading frame revealed a deduced amino acid sequence with 77% identity to the h-BLTR (3). The third intracellular loop of the m-BLTR showed 100% identity to the human receptor, with two protein kinase C phosphorylation sites.

m-BLTR Binding—Because the genomic clone appeared to be intronless, we proceeded directly to expression studies. m-BLTR was subcloned in the expression vector pCR3.1 and used to transfect HEK 293 cells. Transient transfected HEK cells displayed specific binding for LTB₄ (Fig. 1A), as did cells transfected with the original human P2Y₇ receptor clone (not shown), whereas nontransfected (Ct) and mock transfected cells did not. Membrane fractions of HEK-m-BLTR cells showed a reversible, saturable, and high affinity binding for LTB₄ with a Kᵦ = 0.24 ± 0.03 nM and Bₘₐₓ = 743 ± 168 fmol/mg of protein (Fig. 1B). Displacement curves of [³H]LTB₄ binding indicated that the binding site was specific for LTB₄ with a Kᵦ of 0.23 ± 0.05 nM (n = 4), followed by 20-hydroxy-LTB₄, a metabolite of LTB₄ (Kᵦ = 1.1 ± 0.2 nM, n = 4), and by the nonenzymatic breakdown product of LTA₄, 6-trans-12-epi-LTB₄, and LTD₄ (Kᵦ > 1 μM) (Fig. 1C).

Functional Expression of the m-BLT Receptor—The tissue distribution of the m-BLTR was investigated by Northern blot analysis of total RNA using two different probes. No signal was detected in the tissues tested (spleen, lung, kidney, liver, pancreas, uterus, testis, heart, aorta, brain). However, using reverse transcriptase-PCR, the m-BLTR mRNA was detected in all tissues, but not aorta (data not shown). The mouse heart, lung, and serum cDNAs were sequenced and found to be identical with all tissues, but not aorta (data not shown). The mouse heart, lung, and serum cDNAs were sequenced and found to be identical with all tissues, but not aorta (data not shown). The mouse heart, lung, and serum cDNAs were sequenced and found to be identical with all tissues, but not aorta (data not shown). The mouse heart, lung, and serum cDNAs were sequenced and found to be identical with all tissues, but not aorta (data not shown).

Data Analysis—Multiple analysis of variance tests followed by Bonferroni analysis were performed on cAMP data. *p < 0.05; ***p < 0.001.

Fig. 2. m-BLTR-induced pigment aggregation in melanophores. The cloned m-BLTR was transfected into Xenopus melanophores via electroporation. LTB₄ (0.03 pM–10 nM) was added to cells exposed to room light for 1 h. The ordinate scale is explained under “Experimental Procedures.” Each data point is mean ± S.E. of triplicates (n = 3).

Fig. 3. LTB₄ bound to the m-BLTR induces activation of signal transduction pathways in CHO-m-BLTR. Panel A, LTB₄ induced a concentration-dependent inhibition of forskolin (25 μM)-stimulated cAMP accumulation. Different concentrations of LTB₄ (0.01–100 nM) were added to cells stimulated by forskolin (F, 100%). The stimulated level of cAMP by forskolin was 436 ± 83 pmol/10⁶ cells. Data presented are mean ± S.E. (n = 5). Panel B, concentration-dependent increase in intracellular calcium induced by LTB₄. The addition of 1 μM thapsigargin induced the depletion of ER calcium (inset). LTB₄ (100 nM)-induced intracellular calcium increase was inhibited subsequently (n = 4).

X. laevis melanophores provide a rapid, functional and visual readout of receptor activation. These cells disperse their pigment granules upon stimulation of receptors that are coupled to Gₛ, Gₛ, and Gₛ and lead to accumulation of second messengers and thus appear dark. In contrast, stimulation of receptors that are coupled to Gₛ cause a decrease in second messenger levels and result in pigment granule aggregation, and the cells appear light (27). A long-term culture of melanophores was used to evaluate the functional activation of the m-BLTR. Stim-
membranes were prepared and binding of \[3H\]LTB4 examined in gen. Neutrophils were obtained by peritoneal lavage 5 h later. Crude mice (five each) were injected intraperitoneally with 1% sterile glycogen; Fig. 3.

mice does not appreciably alter BLTR binding to neutrophil strains function as coreceptors for more limited numbers of virus chemokine and related orphan receptors have been shown to influence receptor expression, we tested 5-lipoxygenase-deficient mice did not substantially alter BLTR binding (Fig. 4).

With the same cell line, LTB4 induced a concentration-dependent increase in the intracellular calcium levels (EC50 = 4.4 nM; Fig. 3B). With 1 μM thapsigargin, an inhibitor of the endoplasmic reticulum calcium ATPase pump, the intracellular increase of calcium induced by 100 nM LTB4 was inhibited by 89.5 ± 1.4% (n = 4).

BLT Receptor Binding in Leukotriene-deficient Mice—Previously, we (29) and another group (35) developed mice with disruptions in the 5-lipoxygenase gene. These mice were unable to synthesize cysteinyl-leukotrienes or LTB4 in various inflammatory cell types. To test whether the absence of ligand influences receptor expression, we tested 5-lipoxygenase-deficient and control mice for alterations in LTB4 binding using membranes from glycogen-elicited neutrophils. Although we obtained specific and competitive binding in these membranes, the lack of ability to synthesize LTB4 in 5-lipoxygenase deficient mice did not substantially alter BLTR binding (Fig. 4).

Is the BLT Receptor a Coreceptor for HIV/SIV Entry into CD4-expressing Cells?—Primate lentiviruses utilize CD4 and a coreceptor (most often the chemokine receptors CCR5 and CXCR4) to enter target cells (20). The importance of CCR5 as a HIV-1 coreceptor was demonstrated by the finding that individuals who lack CCR5 because of a naturally occurring poly- mutation of the receptor caused pigment aggregation in a concentration-dependent fashion with an EC50 = 0.13 nm (Fig. 2) consistent with coupling to Gi.

In CHO-m-BLTR cells, LTB4 inhibited in a concentration-dependent manner the cAMP production induced by 25 μM forskolin (Fig. 3A). Maximum inhibition (58%) was obtained at 100 nm.

The high level of sequence identity for the m-BLTR gene cloned here with the human sequences R2 (17), CMKRL1 (16), BLTR (3), and the clone classified as a P2Y7 purinergic receptor (19) combined with the binding studies confirm the fact that these are mouse and human orthologs of the BLTR. Additional confirmation comes from a recent independent cloning of m-BLTR cDNA from murine eosinophils (34). LTB4 affinity for m-BLTR was similar to that for h-BLTR (0.24 versus 0.154 nm, respectively). The NH2 terminus as well as the three putative extracellular loops of the receptor show the lowest percentage identity between species (between 25 and 75% identity), in contrast with the high percent identity for the transmembrane domains (between 86 and 95% identity) and the intracellular loops (between 61 and 100%). The third intracellular loop of the m-BLTR, which shows two putative protein kinase C phosphorylation sites, is identical to the human sequence. These phosphorylation sites could be critical in the process of homologous desensitization of the BLTR that has been established in neutrophils (40), eosinophils (41), and granulocytes (42).

The potential protein kinase C as well as the casein kinase 2 (a Ser/Thr protein kinase) phosphorylation sites of the COOH-terminal loop could also be involved in the regulation of the activation/desensitization of the receptor or in the uncoupling of the receptor to G protein.

The rank order of LTB4 binding affinity and of related compounds for the m-BLTR is similar to the h-BLTR (3) and consistent with previous experiments using guinea pig lung membranes (43) and porcine spleen membranes (44). 20-Hydroxy-LTB4, a metabolite of LTB4, was the most potent competitor after LTB4. There was a discrepancy in the efficiency of LTB4 to induce an increase in Ca2+ and to inhibit cAMP production in CHO cells. There was also a distinction in the ability of LTB4 to inhibit cAMP production in CHO cells versus melanomas.

The results described above indicate that the h-BLTR does not serve as a coreceptor for the virus strains tested or does so only inefficiently. We therefore employed a more sensitive cell-cell fusion assay (Fig. 6) in an attempt to detect h-BLTR coreceptor activity. We have found that coreceptors that support virus infection also support Env-mediated cell-cell fusion. However, there are cases when a given Env-coreceptor combination supports cell-cell fusion but does not support virus infection (38, 39).

CD4 and h-BLTRs were coexpressed. Thus, the h-BLTR did not function as a coreceptor for either virus infection or Env-mediated cell-cell fusion.

DISCUSSION

The rank order of LTB4 binding affinity and of related compounds for the m-BLTR gene did not contain a TATA box but revealed the presence of putative consensus sequences for binding various transcription factors, including myeloid-specific factors, which could be implicated in the expression either CCR5 or CXCR4 (depending on the virus strain). In addition to the results depicted in Fig. 5, SIVmac1A11, smPBj6.6, macB670-clone 3, and sm62B were unable to use h-BLTR to infect cells. In separate experiments using transiently transfected HEK 293 cells the number of specific binding sites was found to be approximately 90 fmol/mg of protein.

Although fusion was readily observed with the major HIV-1 coreceptors, fusion using this sensitive assay was not observed when CD4 and h-BLTRs were coexpressed. The BLT receptors did not function as a coreceptor for either virus infection or Env-mediated cell-cell fusion.

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regulation of transcription of the m-BLTR. There was also a repetitive poly(A) sequence whose role remains to be defined.

The alteration of LTB4 synthesis in vivo could modify m-BLTR expression. However, we found that LTB4 binding to neutrophil membranes from 5-lipoxygenase knockout mice, who do not synthesize any leukotrienes, and from wild type mice were similar. In some pathological situations (45, 46), a modification in the level of BLTR binding has been demonstrated. The functional characterization of the promoter region, combined with mutation and deletion studies of this region, would help to elucidate transcription factors involved in the regulation of transcription of m-BLTR, and potentially link the alteration of LTB4 synthesis to inflammatory pathologies where LTB4 is involved. The recent discovery of high expression of m-BLTR in eosinophils (34) will be important to explore in the context of airway inflammation models.

In addition to CCR5 and CXCR4, a host of other chemokine and orphan seven-transmembrane domain receptors have been shown to support infection by smaller numbers of HIV or SIV strains (for review, see Ref. 37). Recently, Owman et al. (22) reported that the h-BLTR functions as an efficient coreceptor for a number of virus strains, especially primary virus isolates that utilize CXCR4. However, we were unable to detect coreceptor activity for any of the 16 SIV and HIV-1 Env proteins examined using either virus infection or a sensitive cell-cell fusion assay with h-BLTR. There are a number of possible explanations for these discrepancies. First, it is simply possible that h-BLTR may function as a coreceptor for the strains tested by Owman et al. (22) but does not function as a coreceptor for the strains tested here. Second, h-BLTR coreceptor function may be dependent upon the cell type or assay used. Owman et al. (22) used a PCR-based entry assay, which may be overly sensitive, whereas we used a reporter virus infection and cell-cell fusion assays. It will be important to demonstrate h-BLTR coreceptor function in stable cell lines using more standard methods of viral quantification, such as p24 measurements. Third, coreceptor function can be highly dependent upon surface expression levels (47, 48). Reagents are not yet available which make it possible to measure BLTR surface expression accurately. It is possible that we expressed suboptimal amounts of plasma membrane BLTR in our system, although this receptor did express well for binding studies in transiently transfected HEK 293 cells. Regardless of the explanation, the importance of h-BLTR as a viral coreceptor will rest upon the number and types of virus strains that can use it for infection, the levels at which it is expressed in vivo, and the CD4-positive cell types in which it is expressed. Only additional work will clarify these points.

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