

Cell-specific Transcriptional Regulation of Human Leukotriene B₄ Receptor Gene

By Kazuhiko Kato,*[§] Takehiko Yokomizo,*[‡] Takashi Izumi,*[‡] and Takao Shimizu*[‡]

From the *Department of Biochemistry and Molecular Biology, Faculty of Medicine, The University of Tokyo, Tokyo 113-0033, Japan; [‡]Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Corporation, Tokyo 113-0033, Japan; and the [§]Pharmaceutical Research Center, Meiji Seika Kaisha, Limited, Yokohama 222-8567, Japan

Abstract

Leukotriene B₄ (LTB₄) is a lipid mediator that activates leukocytes and is involved in host defense and inflammation. BLT1, a high-affinity receptor for LTB₄ (originally termed BLT), is expressed exclusively in inflammatory cells and is inducible in macrophages upon activation. The mechanisms of tissue-specific expression and induction of BLT1 are important for the understanding of mechanism of onset and the potential treatment of inflammatory disorders. Here, we report the genomic structure and a promoter analysis of the human BLT1 gene, with an emphasis on the mechanism of cell-specific transcription. No TATA or CAAT elements exist around the transcription initiation sites, but a GC-rich sequence is observed in this region. A reporter gene assay revealed that a region ~80 basepair upstream from the initiator sequence is required for the basal transcription of the BLT1 gene. Sp1 was found to be a major activator of basal transcription by electrophoretic mobility shift assays and site-directed mutagenesis. The CpG sites of the BLT1 promoter region were highly methylated in BLT1-nonexpressing cells, but not methylated in BLT1-expressing cells. Further, methylation of this region in vitro inhibited the promoter activity to ~15% of the control. Thus, methylation at CpG sites in the promoter region is important for cell-specific transcription of the BLT1 gene. The promoter region of the BLT1 gene is localized within the open reading frame (ORF) of the BLT2 gene, which encodes a low-affinity receptor for LTB₄ (Yokomizo, T., K. Kato, K. Terawaki, T. Izumi, and T. Shimizu. 2000. *J. Exp. Med.* 192:421–431). To our knowledge, this is the first example of “promoter in ORF” in higher eukaryotes.

Key words: leukotriene B₄ receptor • inflammation • methylation • Sp1 • THP-1 cell

Introduction

Leukotriene B₄ (LTB₄),¹ a metabolite of arachidonic acid, is a potent lipid mediator. LTB₄ mainly activates leukocytes, leading to chemotaxis, degranulation, and production of superoxide anions, thus playing important roles in host defense (1, 2). LTB₄ is also related to inflammatory diseases such as rheumatoid arthritis (3), bronchial asthma (4), psoriasis (5), ulcerative colitis (6), and postischemic tissue injuries (7, 8). These actions of LTB₄ are mediated by a

specific cell surface receptor, leukotriene B₄ receptor (BLT). Human BLT1 cDNAs were isolated (9, 10), and its orthologues were obtained from mouse (11, 12), rat (13), and guinea pig (14, 15). Human BLT1 mRNA is expressed abundantly in leukocytes, and to a lesser degree in spleen and thymus. In mice, BLT1 mRNA is abundant in eosinophils isolated from IL-5 transgenic mice and in activated macrophages (11). Enhanced expression was also observed in activated peritoneal macrophages in rats (13). Therefore, the transcription of the BLT1 gene appears to be tightly regulated in a cell- and tissue-specific manner, and is inducible by various stimuli. To elucidate the mechanism of the BLT1 expression, we analyzed the structure, promoter region, and regulation of cell-specific transcription of the human BLT1 gene. During the course of analysis of the BLT1 gene, we identified a putative open reading frame

Address correspondence to Takao Shimizu, Department of Biochemistry and Molecular Biology, Faculty of Medicine, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-0033, Japan. Phone: 81-3-5802-2925; Fax: 81-3-3813-8732; E-mail: tshimizu@m.u-tokyo.ac.jp

¹Abbreviations used in this paper: EMSA, electrophoretic mobility shift assay; GPCR, G protein-coupled receptor; LTB₄, leukotriene B₄; ORF, open reading frame; RACE, rapid amplification of cDNA ends; UTR, untranslated region.

(ORF) in the promoter region for a G protein-coupled receptor (GPCR) with structural similarity to BLT1. This receptor is revealed to be a low-affinity LTB₄ receptor, BLT2, which is reported in an accompanying paper (16).

Materials and Methods

Cell Culture and Isolation of Nucleic Acids. THP-1 (human monocytic leukemia cell line), U937 (human histiocytic lymphoma cell line), and HL-60 (human leukemia cell line) cells were maintained in RPMI 1640 medium. HeLa (human cervical cancer cell line) and HepG2 (human hepatoma-derived cell line) cells were maintained in DME. All media were supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, and 100 µg/ml streptomycin. Poly(A)⁺ RNA was isolated using a QuickPrep Micro Purification Kit (Amersham Pharmacia Biotech). Genomic DNA was isolated by treating cells with 200 µg/ml proteinase K in the presence of 1 mM EDTA and 1% SDS, followed by phenol extraction.

Isolation of the BLT1 Gene. A human lymphocytes genomic library (Stratagene) was screened by plaque hybridization with the BLT1 ORF labeled with α-[³²P]dCTP. The positive genomic clone was subcloned into pBluescript SK(-) and sequenced with an ABI 373 automated sequencer.

5' Rapid Amplification of cDNA Ends Analysis. 5' rapid amplification of cDNA ends (RACE) was performed using a 5' RACE System for Rapid Amplification of cDNA Ends (version 2.0; Life Technologies). 1 µg of poly(A)⁺ RNA prepared from HL-60 cells differentiated by 1 µM retinoic acid for 48 h was used as a template. First-strand cDNA was synthesized with the primer 1 (5'-GAACTCTACACCTAGTGAGG-3' from +48 to +29 relative to the adenosine of first methionine; see Fig. 2 A). PCR was performed using the primer 2 (5'-GTGCTGCAGAAGATGTAGTG-3' from +25 to +6) and the 5' RACE Abridged Anchor Primer (5'-GGCCACGCGTCCGACTAGTACGGGIGGGIIGGGIIG-3'; Life Technologies), followed by nested PCR using the primer 3 (5'-TCCGGACCGTCCTTCTCATCC from -92 to -113 relative to the adenosine of first methionine in HL-5 clone) and the Abridged Universal Amplification Primer (5'-GGCCACGCGTCCGACTAGTAC-3'; Life Technologies). PCR products were subcloned using an Original TA Cloning Kit (Invitrogen), and were sequenced.

Reporter Gene Assay. Various lengths of 5' flanking regions of the BLT1 gene were amplified by PCR using 20 mer of sense and antisense primers. The KpnI and HindIII sites were added to the 5' ends of sense and antisense primers, respectively. These amplified fragments were ligated into the KpnI-HindIII site of the pGL3 basic vector (Promega) and sequenced, and we confirmed that there were no misincorporations by PCR. These 5' flanking region-firefly luciferase gene fusion plasmids (0.5 µg per sample) were transfected into THP-1 (4 × 10⁵ cells per sample) and HeLa cells (4 × 10⁴ cells per sample) using EffecteneTM (QIAGEN). The plasmid, pRL-CMV (Promega; 0.1 µg per sample) containing *Renilla* luciferase gene driven by the cytomegalovirus immediate early enhancer/promoter was cotransfected, and the luciferase activity was normalized. After incubation of the cells at 37°C for 18 h, the luciferase assay was performed using a Dual-Luciferase Reporter Assay System (Promega) and a luminometer (MiniLumat LB 9506; Berthold).

Electrophoretic Mobility Shift Assays. Nuclear extracts were prepared from THP-1 and HeLa cells by the method of Dignam et al. (17). Nuclear extracts containing 5 µg of protein were in-

cubated in 20 µl of binding buffer (10 mM Tris-HCl pH 8.0, 50 mM NaCl, 1 mM MgCl₂, 0.5 mM EDTA, 0.5 mM DTT, 50 µg/ml poly(dI-dC) poly(dI-dC), and 4% glycerol) with or without a cold competitor (a 200-fold molar excess). For supershift assays, 1 µg of anti-Sp1 antibody or rabbit IgG (Santa Cruz Biotechnology, Inc.) was incubated at room temperature for 10 min. The DNA probe (10,000 cpm) labeled with γ-[³²P]ATP was added, and the samples were incubated at room temperature for 20 min. Reaction mixtures were separated in a 4% polyacrylamide gel and autoradiographed to an X-ray film.

Site-directed Mutagenesis. Mutagenesis of the putative Sp1 site in p(-123/+91) was introduced using a QuikChangeTM Site-Directed Mutagenesis Kit (Stratagene) according to the manufacturer's instructions. The primer used were MS-1 (5'-GCCTTG-GCGAAGCTGAACAGAGCCGGCCAGGCGG-3', from -66 to -33 relative to the adenosine of the initiator sequence; mutation sites are shown as bold letters in the primer sequence) and MAS-1 (5'-CCGCCTGGCCGGCTCTGTTTCAGCTTCGC-CAAGG-3', from -33 to -66).

Genomic Southern Blot Analysis. 10 µg of genomic DNAs were digested by HpaII or MspI, then digested by EcoRI. Reaction mixtures were separated in 1% agarose gels and transferred to Hybond N⁺ membranes (Amersham Pharmacia Biotech). The membranes were incubated with α-[³²P]dCTP labeled DNA probes (see Fig. 5 C) at 65°C overnight, and washed with 0.1 × SSC, 0.1% SDS at 65°C. The washed membrane was autoradiographed to an X-ray film.

Effect of Methylation at CpG Sites on the Promoter Activity. 20 µg of the plasmid p(-123/+91) was digested by KpnI and HindIII, and the insert was purified from a 2% agarose gel. This fragment was incubated with or without SssI methylase (6 U/µg DNA; New England BioLabs, Inc.) at the presence of 16 µM S-adenosylmethionine. After incubation at 37°C for 3 h, the DNA fragment was ethanol precipitated, followed by ligation to the pGL3 basic vector at 16°C for 16 h. Aliquots of the ligated DNA were ethanol precipitated and transfected into HeLa cells, and the luciferase assay was performed.

Results

Isolation of the BLT1 Gene. ~10⁶ genomic clones were screened using the human BLT1 ORF as a probe, and we obtained one positive clone (LambdaNOK) containing the BLT1 gene. The map of this clone is shown in Fig. 1. The human BLT1 gene is ~5.5 kb in length and consists of three exons. Most of the 5' untranslated region (UTR) of either the HL-5 or HL-1 clone (10) is present on exon I and exon II, respectively. The BLT1 ORF is found on exon III, and is not interrupted by any introns. All the exon/intron junctions obeyed the rule of GT/AG consensus sequence (data not shown; 18).

5' RACE Analysis. When nested PCR was performed using an oligonucleotide specific to the HL-5 sequence (primer 3; Fig. 2 A), several amplified fragments were observed (data not shown). Sequence analysis of these fragments revealed three transcription initiation sites (Fig. 2 B, asterisks). However, using a primer specific to the HL-1 clone, no specific fragment was observed (data not shown). Nucleotide sequences of the putative promoter region are shown in Fig. 2 B. One of the transcription initiation sites is highly homologous to the initiator sequence

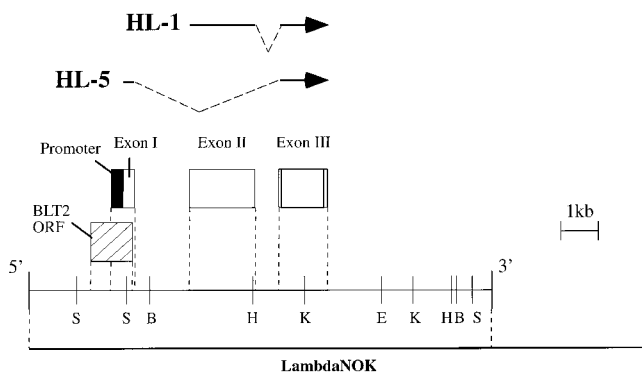


Figure 1. Genomic structure of the human BLT1 gene. The ORF (light gray box), promoter region (dark gray box), and UTRs (open boxes) of the BLT1 gene and the ORF of the BLT2 gene (hatched box) are indicated. Restriction enzyme sites are indicated as follows: B, BamHI; E, EcoRI; H, HindIII; K, KpnI; and S, SacI. These sequence data (LambdaNOK) are available from EMBL/GenBank/DBJ under accession no. AB008193. Note that the BLT2 ORF is overlapped with the BLT1 promoter region.

(YYA⁺NT/AYY, where Y is a pyrimidine nucleotide; reference 19). Neither a TATA box nor a CAAT box was observed near these transcription initiation sites, but a high GC content was observed in this region. A search for binding sites for transcription factors revealed consensus sequences for NFκB (20), USF (21), AP-1 (22), AP-4 (23), and Sp1 (reference 24; Fig. 2 B).

Promoter Activity and ORF in the Promoter Region. The promoter activity was determined by transfecting the 5' flanking region–luciferase gene fusion plasmids to THP-1 and HeLa cells. THP-1 cells express BLT1, whereas HeLa cells do not (see Fig. 5 D). Significant promoter activity was observed in both cell lines transfected with the constructs containing the region between –1091 and –76 relative to the adenosine residue of the initiator sequence (Fig. 3). This activity was decreased markedly when the region between –76 and –33 was deleted. No activity was detected using p(–4/+91) in both cells. These results suggest that the region from –76 to –33 is crucial for the basal transcription of the BLT1 gene. Surprisingly, another ORF homologous to BLT1 overlapped the promoter region. This ORF was shown to encode the second receptor of LTB₄, which is described in detail in an accompanying paper (16).

Electrophoretic Mobility Shift Assays and Site-directed Mutagenesis. By luciferase assay, the region from –76 to –33 was found to be an important cis element in the basal transcription of the BLT1 gene. Between –76 and –33, there is a consensus sequence for Sp1 binding (Fig. 2 B). Electrophoretic mobility shift assay (EMSA) was performed to determine whether Sp1 binds to this region. Several DNA–protein complexes were observed in nuclear extracts of both THP-1 and HeLa cells (Fig. 4 A, lanes 2 and 8). The upper two bands disappeared when an excess of unlabeled –76/–33 (a 200-fold molar excess) was added (Fig. 4 A, lanes 3 and 9), showing that these bands are specific for this

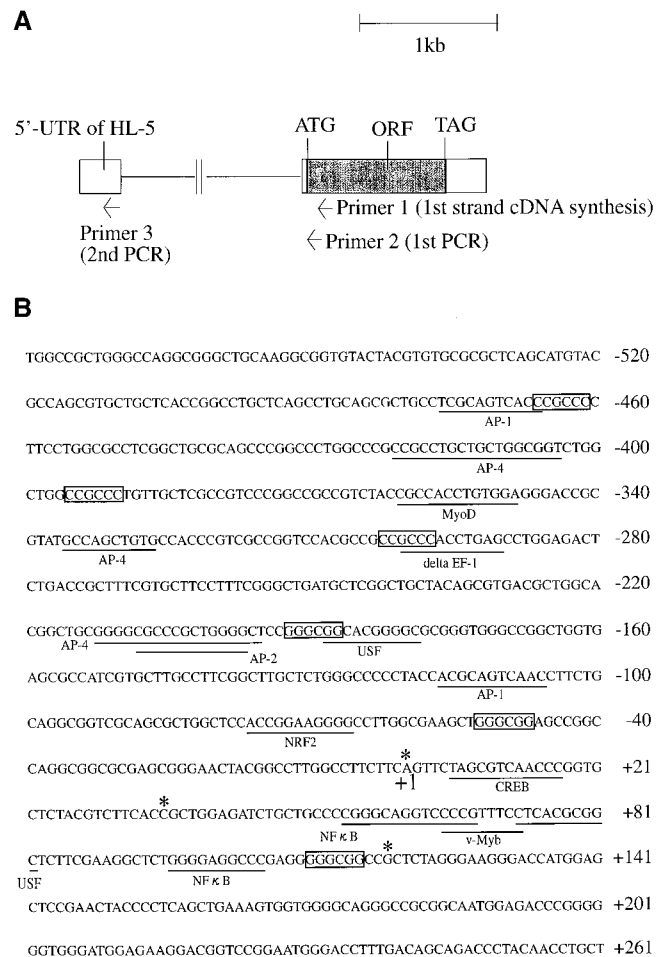


Figure 2. Identification of the transcription initiation site of the BLT1 gene. (A) The sites of primers used for 5' RACE are shown. Arrows indicate the synthetic oligonucleotides. (B) Nucleotide sequences of the 5' flanking region of the BLT1 gene. Asterisks show the transcription initiation sites determined by 5' RACE. GC boxes (consensus sequences of Sp1) are shown in open boxes. The consensus sequences of various transcription factors are underlined.

region. However, an excess of oligonucleotide mutated at this putative Sp1 binding site (5'-GAACAG-3' at –52 to –47; mutation sites are shown as bold letters) did not compete away the binding (Fig. 4 A, lanes 4 and 10). Coincubation with anti-Sp1 antibody resulted in a supershift (Fig. 4 A, lanes 6 and 12), but control IgG did not give this response (Fig. 4 A, lanes 5 and 11). These results show that Sp1 binds to the GC box at –52/–47 in the BLT1 promoter region. Moreover, a lower band was supershifted when an Sp3 antibody was coincubated (data not shown). To investigate the effect of Sp1 binding in the promoter activity, site-directed mutagenesis was performed. In both THP-1 and HeLa cells, the luciferase activity of the mutated construct was decreased to ~25% of the wild-type (Fig. 4 B). These results indicate that Sp1 binds to the BLT1 promoter region and activates the basal transcription.

Methylation of CpG Sites in the Promoter Region. There are many CpG sites surrounding the promoter region of

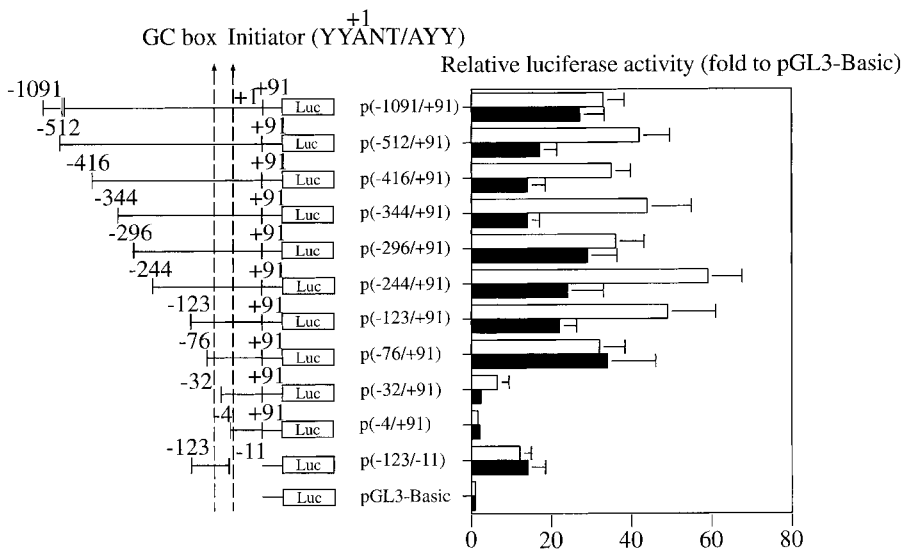


Figure 3. Serial deletion mutant analysis of the BLT1 promoter. Promoter activities are shown as the luciferase activity relative to that of the pGL3 basic vector (a promoterless vector). The activities of THP-1 cells (white bars) and HeLa cells (black bars) are shown as the mean \pm SD from three independent experiments performed in triplicate.

the BLT1 gene. To determine the methylation state at these CpG sites, Southern blotting was performed using genomic DNAs from various cell lines. Although both MspI and HpaII recognize and digest 5'-CCGG-3' sequences, MspI but not HpaII can cleave these sequences when the second cytosine residue is methylated. Capitalizing on this difference, methylation in the promoter region was detected. Using probe A, a 5' flanking region of the promoter, a band of 1.1 kb was detected by MspI digestion in all the cells examined (Fig. 5 A). However, this band of 1.1 kb was detected only in HL-60 and U937 cells by

HpaII digestion, whereas a partial digested band of 2.5 or 5.5 kb was detected in HL-60, U937, and THP-1 cells or HL-60 cells, respectively. In HeLa and HepG2 cells, neither the 1.1 kb nor the 2.5 kb band was observed, whereas longer bands (\sim 3.0 kb and 7.0 kb, respectively) were detected (Fig. 5 A). Similar results were observed in Southern blotting using probe B, a sequence 3' to the promoter region (Fig. 5 B). Considering the size of the detected bands, the region surrounding the BLT1 promoter is not methylated in U937 and THP-1 cells, and is almost completely methylated in HeLa and HepG2 cells. In HL-60 cells (both

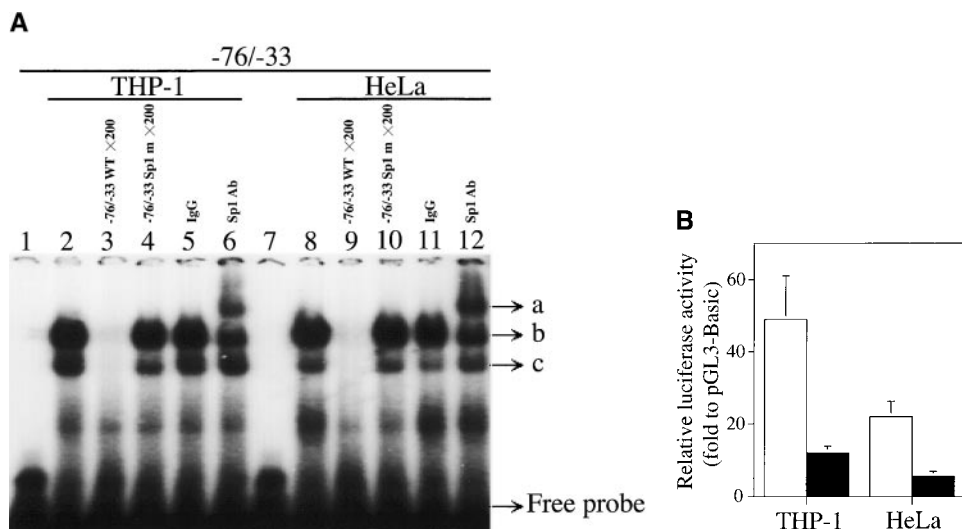


Figure 4. Sp1 binds and activates the BLT1 promoter. (A) EMSAs were performed with 32 P-labeled -76/-33 using nuclear extracts prepared from THP-1 and HeLa cells. Two DNA-protein complexes were detected in both cells (lanes 2 and 8; shown as b and c). They were competed by 200-fold molar excess of unlabeled -76/-33 (-76/-33 WT, lanes 3 and 9), and not by 200-fold molar excess of unlabeled -76/-33 with mutations in the GC box (-52/-47) (-76/-33 Sp1 m, lanes 4 and 10). The band b was supershifted by incubation with 1 μ g of anti-Sp1 antibody (lanes 6 and 12; shown as a) but not by the control IgG (lanes 5 and 11). (B) The effect of mutagenesis of Sp1 binding site on the promoter activities. Luciferase activities of the wild-type construct, p(-123/+91) (white columns) and the construct mutated at GC box (black columns) are indicated as mean \pm SD from three independent experiments performed in triplicate. Mutation sites are shown in bold at right.

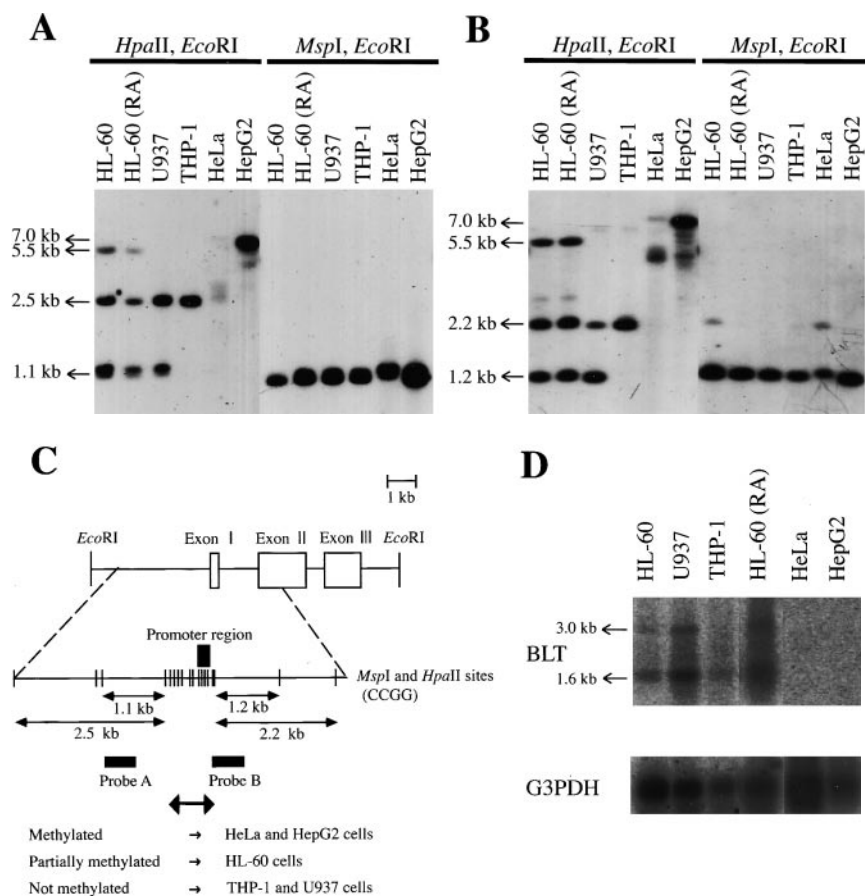


Figure 5. Methylation of CpG sites in the BLT1 promoter region. (A and B) Genomic DNAs isolated from various cell lines were digested with *HpaII* or *MspI*, followed by digestion with *EcoRI*. The digested DNAs were electrophoresed in 1% agarose gels, transferred to nylon membranes, and blotted with (A) probe A and (B) probe B. The positions of the probes A and B are shown in C. HL-60 (RA) means HL-60 cells differentiated by 1 μ M retinoic acid for 48 h. (C) Methylation sites and genomic organization. (D) Northern blotting of various cells for BLT1 ORF. 3 μ g of poly(A)⁺ RNA was used for each lane.

differentiated and nondifferentiated), this region appears to be partially methylated. Northern blotting of these cell lines showed that HL-60, U937, and THP-1 cells express BLT1 mRNA, whereas HeLa and HepG2 cells do not (Fig. 5 D). These results led us to the conclusion that methylation inhibits BLT1 transcription. Thus, the effect of methylation on the promoter activity was investigated. The insert of p(-123/+91) was treated with *SssI* methylase, which methylates cytosine residues at the CpG sites, and a luciferase assay was performed. The activity of methylated construct was decreased to ~15% of that of the unmethylated construct (Fig. 6), supporting the conclusion that methylation of the CpG sites inhibits the BLT1 promoter activity.

Discussion

LTB₄ is a potent activator of granulocytes, eosinophils, and macrophages. The biological activities of LTB₄ are comparable to those of IL-8 and FMLP, so-called "classical chemoattractants" (25). LTB₄ is unique because it is a lipid mediator biosynthesized from membrane phospholipids by the actions of phospholipase A₂ (26, 27), 5-lipoxygenase, and LTA₄ hydrolase (2, 28). The actions of LTB₄ are believed to be mediated by a specific cell surface receptor, BLT (29). Mice overexpressing human BLT1 (originally

termed BLT) showed enhanced granulocyte accumulation in skin microabscesses and lungs after ischemia reperfusion induced tissue injury (30), confirming the pathophysiological importance of LTB₄ in vivo.

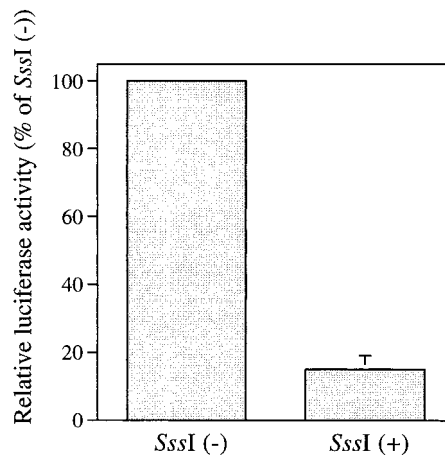


Figure 6. Effect of the methylation at CpG sites on the promoter activity. The insert of p(-123/+91) was incubated with or without *SssI* methylase, ligated into pGL3 basic vector, and transfected into HeLa cells. The relative luciferase activities are shown as mean \pm SD from three independent experiments performed in triplicate.

The cDNA cloning of an LTB₄ receptor, BLT1, showed that BLT1 is a GPCR with seven transmembrane domains with few similarities to known receptors, including those for chemoattractants and prostanoids (10). We and others have successfully cloned cDNA and genomic DNAs of BLT1 from other species, including mouse (11, 12), guinea pig (14, 15), and rat (13), and have shown that the primary structures of BLT1 are conserved among species especially in the TM domains. BLT1 isolated from various animals showed subnanomolar K_d values for ³H-LTB₄ in transfection studies, and the binding was inhibited by a number of specific BLT antagonists. Therefore, BLT1 is a high-affinity receptor for LTB₄. The distribution of BLT1 is restricted, with expression observed almost exclusively in peripheral leukocytes (10, 14), activated macrophages (13), and eosinophils (11).

In this manuscript, genomic structure, characterization of the promoter region, and mechanism of cell-specific transcription of the BLT1 gene are reported. The BLT1 gene consists of three exons, and the ORF is intronless like other receptors for chemoattractants such as FMLP (31), IL-8 (32), and platelet-activating factor (PAF [33]). Owman et al. (9) have shown that the human BLT1 gene is localized in 14q11.2-q12. Three transcription initiation sites were detected by 5' RACE analysis when the primer specific to the 5' UTR of HL-5 clone was used, and these sites were scattered over 120 bp. There are no TATA or CAAT elements around these initiation sites, but one of initiation sites is highly homologous to the initiator sequence. The initiator sequence has been reported to play a role in transcription initiation of various genes (19, 33). No transcription initiation site was detected using the primer specific to the 5' UTR of HL-1 clone. Two human BLT1 cDNAs, HL-1 and HL-5, were isolated, and were shown to share the same ORF, but different 5' UTRs (34). In Northern blotting of HL-60 and U937 cells, the probe containing the 5' UTR specific to HL-5 clone gave positive signals of ~1.6 kb and 3.0 kb (data not shown), which were similar to the results from the probe of the entire ORF (Fig. 5 D; reference 10). In contrast, the probe containing the 5' UTR specific to HL-1 clone gave no signals on the same membrane (data not shown), suggesting that HL-5 and not HL-1 is the major transcript of BLT1, at least in HL-60 and U937 cells.

We next examined the BLT1 promoter activity using the 5' flanking region-luciferase fusion plasmids. The results from various truncated mutants showed that the region between -76 and the initiator sequence is required for the basal transcription of the human BLT1 gene. EMSA analysis revealed that Sp1 binds to -52/-47 of the BLT1 promoter. By introducing the mutation at this Sp1 binding site in p(-123/+91), the promoter activity was decreased to ~25% of the wild-type. These results clearly indicate that Sp1 is important for the basal transcription of the human BLT1 gene. In the promoter of several myeloid-specific genes such as CD11b (35), CD18 (36), and hematopoietic cell kinase genes (37), Sp1 was reported to act as a major activator. Although Sp1 is a ubiquitous transcription

factor, it is preferentially expressed in hematopoietic cells (38). It is still undetermined how Sp1 mediates the tissue-specific transcription. In our study, significant basal promoter activity was observed in HeLa cells, which do not express BLT1 intrinsically. EMSA showed that Sp1 binds to the BLT1 promoter using nuclear extracts from HeLa cells, and mutagenesis in this Sp1 binding site abolished the promoter activity in HeLa cells, as observed in the case of THP-1 cells. Therefore, Sp1 binding to the BLT1 promoter clearly can not explain the tissue- and cell-specific expression of BLT1. The promoter analysis of l-histidine decarboxylase (39) and leukosialin (CD43 [reference 40]) genes revealed that Sp1 is a major activator for their transcription. The methylation of their promoter regions at CpG sites plays an important role in cell- and tissue-specific transcription. As the promoter region of BLT1 gene has high CpG contents with Sp1 as a major activator, we studied the methylation state of the region surrounding the BLT1 promoter in BLT1-expressing and -nonexpressing cells.

Genomic Southern blotting was performed to investigate the methylation state in various cell lines. BLT1-expressing cells showed the shorter positive bands by digestion with HpaII, which can cleave only unmethylated CCGG sequences. In contrast, HeLa and HepG2 cells, which do not express BLT1, exhibited the longer digested bands by HpaII. This implies the significant correlation between the expression of BLT1 and the methylation state. Next, we methylated the BLT1 promoter construct p(-123/+91) by SssI methylase *in vitro*, and examined the effect of this methylation on the promoter activity in HeLa cells. The methylated construct showed only 15% promoter activity of the unmethylated construct. These results show that the cell-specific expression of BLT1 is primarily dependent on the methylation of the promoter region. However, EMSA showed that Sp1 binds similarly to methylated and unmethylated DNA probes (-76/-33) in THP-1 and HeLa cells (data not shown). Sp1 is known to bind to the GC box in a methylation-insensitive manner (41). The methyl-CpG binding proteins, MeCP1 (42) and MeCP2 (43), have been reported to interact specifically with methylated DNA and repress the transcription. These MeCPs might play a role as a repressor in the BLT1 transcription.

Furthermore, we found another ORF overlapping the promoter and 5' UTR of the BLT1 gene. This gene has high homology with BLT1 and encodes a novel GPCR. We have named this GPCR as BLT2, and have reported the role as a low-affinity receptor for LTB₄ (16). In prokaryotes, the overlapping of the promoter and ORF has been reported (44). For example, in *Escherichia coli*, the promoter of the *b*-lactamase gene (*ampC*) is located within the last structural gene of the fumarate reductase (*frd*) operon, and the *ampC* attenuator served as the terminator for transcription of the *frd* operon (45). In our case, the expression pattern of BLT1 and BLT2 is partially overlapped at tissue level, as observed in Northern blotting (10, 16). However, the biological significance of the overlapping of the promoter and ORF was not clarified. Further study should be

needed to demonstrate the significance of gene organization of two related receptors, BLT1 and BLT2. To our knowledge, this is the first example in mammals that the ORF localizes within the promoter region of another gene, so called "promoter in ORF."

In conclusion, we have determined the genomic structure of the human BLT1 gene, and have also identified the transcription initiation sites and the promoter region of this gene. Sp1 binding to the promoter region was required for the basal transcription of the BLT1 gene. Although the precise mechanism of the tissue-specific transcription of the BLT1 gene is still not clear, we found that the CpG island of the BLT1 promoter is methylated in the cells that do not express BLT1, and also that this methylation inhibits BLT1 transcription *in vitro*. Our findings enhance the understanding of how genes are transcribed in a cell- and tissue-specific manner, and assist the understanding of the pathogenesis of inflammatory diseases in which LTB₄ and LTB₄ receptors are involved. In addition, the presence of "promoter in ORF" in the human genome promises to provide insights into how mammalian genes show flexibility and complexity in the transcriptional regulation.

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