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Subject: Progress Report for Moran Foundation Project 97-0089

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Mouse leukotriene A_4 hydrolase is expressed at high levels in intestinal crypt cells and splenic lymphocytes

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Mouse leukotriene A_4 hydrolase is expressed at high levels in intestinal crypt cells and splenic lymphocytes

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Abstract

LTA_4 hydrolase (EC 3.3.2.6) is a dual-function enzyme that is essential for the conversion of leukotriene A_4 (LTA_4) to leukotriene B_4 (LTB_4) and also possesses an aminopeptidase activity. To characterize the expression of this unusual enzyme, we have cloned the mouse LTA_4 hydrolase cDNA. The deduced amino acid sequence revealed 92% identity with the human sequence. Cloning and analysis of genomic sequences of mouse LTA_4 hydrolase indicated that it is a single-copy gene spanning over 40 kb and containing 20 exons. LTA_4 hydrolase is widely expressed, with the highest levels of expression occurring in the small intestine, followed by the spleen. In situ hybridization revealed that LTA_4 hydrolase is localized in the crypt cells of the small intestine, white pulp of the spleen, bronchiolar epithelium of the lung, myocardium, adrenal cortex, epithelium of the seminal vesicles, proximal tubules and the collecting ducts of the kidney, and occasional hepatocytes. Thus the widespread distribution of LTA_4 hydrolase in various cell types in the tissues suggests that LTB_4 may possess biological activities other than those known at present. It is also plausible that the widespread occurrence of LTA_4 hydrolase in various tissues may correspond more with its function as an aminopeptidase than its function as an LTA_4 hydrolase.

Keywords: Amino acid sequence; Eicosanoid; Epoxide hydrolase; In situ hybridization; Recombinant DNA

1. Introduction

Leukotriene A_4 (LTA_4) hydrolase (EC 3.3.2.6) is a key cytosolic enzyme in the arachidonic acid cascade that catalyzes the conversion of the unstable epoxide intermediate LTA_4 into the potent proinflammatory molecule leukotriene B_4 (LTB_4) (Borgeat and Samuelsson, 1979; Radmark et al., 1984). It is also a bifunctional enzyme that possesses intrinsic aminopeptidase activity. The biological substrates of this enzymatic activity have not been identified and its physiological significance is not well understood (Haggstrom et al., 1990; Minami et al., 1990; Orning et al., 1994). Catalysis by LTA_4 hydrolase is considered to be the rate-limiting step in the generation of LTB_4, as the enzyme is known to be suicide inactivated by its substrate LTA_4 (McGee and Fitzpatrick, 1986; Orning et al., 1992). Leukotrienes are a family of oxygenated metabolites derived from arachidonic acid derivatives that potently mediate a variety of physiological and pathophysiological processes (Henderson, 1994; Goetzl et al., 1995; Serhan et al., 1996). They comprise both the cysteinyl leukotrienes C_4, D_4, and E_4 and the noncysteinyl LTB_4 that are implicated in acute inflammatory and immediate hypersensitive reactions and in normal biological homeostatic responses (Henderson, 1945; Boone et al., 1993; Stenke et al., 1993; Peppelenbosch et al., 1992). Of these, LTB_4 is the most potent chemotactic and chemokinetic substance for the neutrophils (Henderson, 1994). It is believed to stimulate adhesion of circulating neutrophils to vascular endothelium and promote their migration to the sites of inflammation and the release of granular constituents (Goetzl and Sun, 1979; Palmer et al., 1980; Shalati et al., 1981). Elevated levels of LTB_4 have been reported in patients with glomerulonephritis, rheumatoid arthritis, psoriasis, and inflammatory bowel disease (Lewis et al., 1990).

Abbreviations: LTA_4, leukotriene A_4; LTB_4, leukotriene B_4; RT-PCR, reverse transcription–polymerase chain reaction; UTR, untranslated region.

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During the last decade, LTA_4 hydrolase-encoding cDNAs have been cloned from several mammalian species, including rat and humans (Makita et al., 1992; Funk et al., 1987). The human gene for LTA_4 hydrolase has been cloned and characterized (Mancini and Evans, 1995). In view of the importance of LTA_4 hydrolase in leukotriene metabolism, more information about its expression would be helpful. In particular, the cloning and characterization of the mouse LTA_4 hydrolase gene may allow further insights into the transcriptional regulation of LTA_4 hydrolase and the precise cellular localization of its synthesis.

We report here the isolation and characterization of both the cDNA and gene for mouse LTA_4 hydrolase and a comprehensive cellular localization of its expression. We also report the results of our comparison of the amino acid sequences of mouse and human LTA_4 hydrolase and our structure analysis of the mouse gene. By Northern blot analysis, we found the enzyme to be widely expressed in mouse tissues.

2. Materials and methods

2.1. Reverse transcription–polymerase chain reaction (RT–PCR)

To generate LTA_4 hydrolase cDNA probes for screening, four pairs of degenerate oligonucleotide primers based upon published LTA_4 hydrolase cDNA sequences (Funk et al., 1987; Makita et al., 1992) were synthesized. They were as follows: primer 1, 5′-CMGAGRTMG-TGGATACCTGTTC-3′ (sense); primer 2, 5′-AGAT-AYGGGTGTTCCT TCCCAG-3′ (antisense); primer 3, 5′-GACSTCTCCAAAATCTTCTGC-3′ (sense); primer 4, 5′-GTAAGYCAKGGYTTCTCCATG-3′ (antisense); primer 5, 5′-CACCATCC TTCYCTTAYGGYGY-3′ (sense); primer 6, 5′ TATGGGAGGCAGTCCMGGR GA-3′ (antisense); primer 7, 5′-ACTCYCCKGGACTG-CCTCCCATA-3′ (sense); primer 8, 5′-RTCATGGGA-TTTGTCAAAGGC-3′ (antisense). Please note that in the primer sequences, K denotes T+G, M stands for A+C, R, S denote A+G and C+G, respectively, and Y represents C+T. Using the antisense primers, approx. 2.5 μg of total RNA from mouse C57 BL/6 lung was reverse transcribed by avian myeloblastosis virus reverse transcriptase. The resultant cDNAs were amplified by PCR using a Perkin Elmer Cetus DNA model 480 thermal cycler under the following conditions: 35 cycles at 94°C for 1 min; 55°C for 1 min; and 72°C for 2 min. The PCR products corresponding to the different regions of the cDNAs were then gel purified and cloned into pT7 Blue T-Vectors (Novagen, Madison, WI). The cloned DNAs were verified by sequencing.

2.2. Northern blot analysis of mouse RNA

Total RNAs were prepared from various mouse tissues using the guanidinium thiocyanate procedure (Chomczynski and Sacchi, 1987). 15 μg of total RNA from these tissues were electrophoresed on a 1% agarose, 2.2 M formaldehyde gel; transferred to a Zeta-Probe nylon membrane (Bio-Rad); hybridized with a 390 bp ^{32}P-labeled cDNA probe corresponding to the 3′-region of the mouse LTA_4 hydrolase cDNA (this paper); washed; and autoradiographed as described earlier (Habib et al., 1998).

2.3. In situ hybridization

Sequences representing 460 bp of the middle region of mouse LTA_4 hydrolase cDNA were amplified by RT–PCR using mouse lung RNA as the template, primer 3 (see above) as the sense primer, and primer 4 (see above) as the antisense primer. This fragment was then cloned into a pT7 Blue T-Vectors in both orientations.

Riboprobes were generated by transcribing the clones in both orientations from *Bam*HI-linearized templates using T7 RNA polymerase in the presence of digoxigenin-labeled UTP (a labeling mixture obtained from Boehringer Mannheim). Typically, 1 μg of template yielded 4–6 μg of riboprobe. No specific labeling was seen using a sense RNA probe. Paraffin-embedded sections were mounted on RNase-free coated slides, prehybridized, hybridized, and washed as reported earlier (Habib et al., 1996).

2.4. Southern blot analysis of mouse genomic DNA

Mouse genomic DNA (15 μg) from kidney was digested with different restriction endonucleases, separated on a 1% agarose gel, and electrophoretically transferred onto a Zeta-Probe nylon membrane. The filter was then hybridized to a ^{32}P-labeled cDNA probe corresponding to the 3′ region of LTA_4 hydrolase cDNA and washed under the same conditions as described earlier (Habib et al., 1996).

2.5. Genomic library screening

A mouse 129 SvEv liver genomic library in a λ Fix II Vector (Stratagene Cloning) Systems, La Jolla, CA, was screened using ^{32}P-labeled cDNA probes corresponding to the entire coding region of the LTA_4 hydrolase cDNA. The genomic library was plated at approx. 50 000 plaques per 150 mm dish and the filter lifts were hybridized in duplicate to the probes under the following conditions: First, hybridization was performed overnight at 65°C in 6×SSC, 0.25% Carnation nonfat dry milk, 100 μg/ml denatured salmon sperm DNA, and 10^6 cpm/ml of [α-^{32}P]dATP-labeled LTA_4

hydrolase cDNAs. Then, the filters were washed with 2 × SSC and 1% SDS, followed by 0.2 × SSC and 0.1% SDS, at 65°C. After three rounds of screening, pBluescript SK phagemids were excised in vivo from plaque-purified phages according to the manufacturer's recommendations. Finally, phage DNA was prepared essentially as described previously (Chisholm, 1989).

2.6. Restriction mapping and DNA sequence analysis

Phage DNA samples were digested with various restriction enzymes under single- and double-digestion conditions and then run on a 1% agarose gels. Ethidium bromide-stained gels were photographed under UV light, and the DNA was blotted onto nylon membranes as described above for Southern blot analyses. Hybridizations were carried out with appropriately labeled mouse LTA_4 hydrolase cDNA fragments, also as described above. Appropriate positive restriction fragments were isolated from 1% agarose gels and subcloned into the pBluescript SK+/− Vector (Stratagene).

Double-stranded plasmid DNAs derived from genomic and cDNA subclones were sequenced using an automated Applied Biosystems model 373 DNA sequencer employing the Applied Biosystems dideoxy terminator cycle sequencing kit (Perkin Elmer Cetus, Foster City, CA). Universal primers such as T3 and T7 promoter primers and synthetic primers designed based on the mouse LTA_4 hydrolase cDNA sequence were used for sequencing. Sequence alignment and comparison were done using the Intelligenetics (IG) suite (Intelligenetics, Palo Alto, CA).

2.7. Construction of mouse lung LTA_4 hydrolase cDNA clones by RACE PCR

Mouse lung poly(A)$^+$ RNA (1 μg) and a Marathon cDNA Amplification Kit (Clontech Laboratories, Palo Alto, CA) were used to generate 5′ ends of LTA_4 hydrolase cDNAs. The synthesis of double-stranded cDNA and anchor ligation were done essentially according to the manufacturer's instructions. The ancho-ligated cDNA was then amplified using anchor primer 1 (AP_1) and LTA_4 hydrolase-specific complementary primer 9 (5′-GCAGACGGTAGCCGGTGAAGC-3′). The product obtained from this amplification was then diluted and reamplified using nested anchor primer 2 (AP_2) and nested LTA_4 hydrolase-specific complementary primer 10 (5′-TAATGAACAGGTATCCA CGACC-3′). To generate 3′ ends, the anchor-ligated cDNA was amplified using sense primer 11 (5′-AC-GCCCTTTATTCAAGGACCTGG-3′) and AP_1. The amplified product was then diluted and reamplified using nested sense primer 12 (5′-CCGCCTTTGACAAA TCCCATGAC-3′). The amplified products were then separated on 1% agarose gels, and the band corresponding to 200–600 bp was cut out, purified, and subcloned into pT7 Blue T-Vector. The clones that had inserts were then sequenced using T7 or puc/M13 forward primers as described above.

3. Results and discussion

3.1. Isolation and sequencing of LTA_4 hydrolase cDNA

Utilizing a combination of degenerate reverse transcription–polymerase chain reaction (RT–PCR) and rapid amplification chain end (RACE) PCR, we cloned a cDNA encoding mouse LTA_4 hydrolase from mouse lung. Our approach was to make several combinations of degenerate primers based on the published sequences of human and rat cDNAs (Funk et al., 1987; Makita et al., 1992). The five overlapping cDNA fragments that were obtained by amplification were sequenced to confirm their identity. The sequences encompassing the 5′ and 3′ ends were obtained by RACE amplification. We found two different sets of sequences in the 5′ untranslated region (UTR) in our RACE clones, which we have designated types I and II (Fig. 1). Type I clones consist of 165 nucleotides of 5′ UTR, 1836 nucleotides of the coding region and 67 nucleotides of 3′ UTR (15 clones). Type II clones consisted of an identical coding region and 3′ UTR, but a unique 5′ end of 57 bases (two clones). Both types of 5′ ends joined the cDNA at position −18. The 3′ UTR had a poly(A) stretch but no polyadenylation signal, as is the case for human and guinea-pig cDNAs (data not shown). The cloned sequence contained an open reading frame encoding 611 amino acids following an ATG start codon. The molecular weight of the sequence was estimated to be 68 906 (Fig. 2). Comparison of the mouse LTA_4 hydrolase amino acid sequence with the human sequence revealed a high degree of identity (92%), with a one-amino-acid (histidine) gap at position 518. The overall identity between mouse and human at the nucleic acid level was 85% (data not shown).

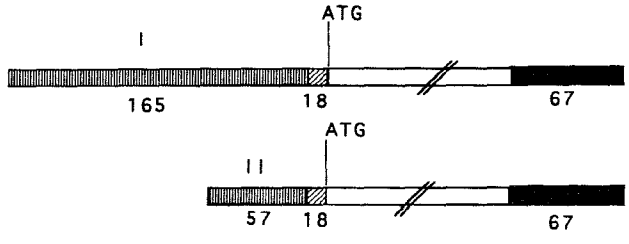

Fig. 1. Organization of the 5′ ends of mouse LTA_4 hydrolase mRNA. Schematic representation of the two mRNAs with different 5′-untranslated regions obtained from mouse lung RACE libraries. Vertical hatched boxes indicate the unique 5′-untranslated regions, hatched boxes indicate the common untranslated region, open boxes indicate the coding region (not drawn to scale), and solid boxes indicate the 3′ untranslated region.

```
mouse  MPEVVDTCSLASPASVCRTQHLHLRCSVDFARRTLTGTAALTVQSQEENLRSLTLDTKDLTIEKVVINGQEV   72
human  MPEIVDTCSLASPASVCRTKHLHLRCSVDFTRRTLTGTAALTVQSQEDNLRSLVLDTKDLTIEKVVINGQEV   72

mouse  KYTLGESQGYKGSPMEISLPIALSKNQEIVIEISFETSPKSSALQWLTPEQTSGKEHPYLFSQCQAIHCRAI  144
human  KYALGERQSYKGSPMEISLPIALSKNQEIVIEISFETSPKSSALQWLTPEQTSGKEHPYLFSQCQAIHCRAI  144

mouse  LPCQDTPSVKLTYTAEVSVPKELVALMSAIRDGEAPDPEDPSRKIYRFNQRVPIPCYLIALVVGALESRQIG  216
human  LPCQDTPSVKLTYTAEVSVPKELVALMSAIRDGETPDPEDPSRKIYKFIQKVPIPCYLIALVVGALESRQIG  216

mouse  PRTLVWSEKEQVEKSANEFSETESMLKIAEDLGGPYVWGQYDLLVLPPSFPYGGMENPCLTFVTPTLLAGDK  288
human  PRTLVWSEKEQVEKSAYEFSETESMLKIAEDLGGPYVWGQYDLLVLPPSFPYGGMENPCLTFVTPTLLAGDK  288

mouse  SLSNVIAHEISHSWTGNLVTNKTWDHFWLNEGHTVYLERHICGRLFGEKFRHFHALGGWGELQNTIKTFGES  360
human  SLSNVIAHEISHSWTGNLVTNKTWDHFWLNEGHTVYLERHICGRLFGEKFRHFNALGGWGELQNSVKTFGET  360

mouse  HPFTKLVVDLKDVDPDVAYSSIPYEKGFALLFYLEQLLGGPEVFLGFLKAYVEKFSYQSVTTDDWKSFLYSH  432
human  HPFTKLVVDLTDIDPDVAYSSVPYEKGFALLFYLEQLLGGPEIFLGFLKAYVEKFSYKSITTDDWKDFLYSY  432

mouse  FKDKVDLLNQVDWNAWLYAPGLPPVKPNYDVTLTNACIALSQRWVTAKEEDLSSFSIADLKDLSSHQLNEFL  504
human  FKDKVDVLNQVDWNAWLYSPGLPPIKPNYDMTLTNACIALSQRWITAKEDDLNSFNATDLKDLSSHQLNEFL  504

mouse  AQVLQKRAPLPLG-IKRMQEVYNFNAINNSEIRFRWLRLCIQSKWEEAIPLALKMATEQGRMKFTRPLFKDL  575
human  AQTLQKRAPLPLGHIKRMQEVYNFNAINNSEIRFRWLRLCIQSKWEDAIPLALKMATEQGRMKFTRPLFKDL  576

mouse  AAFDKSHDQAVHTYQEHKASMHPVTAMLVGRDLKVD                                      611
human  AAFDKSHDQAVRTYQEHKASMHPVTAMLVGKDLKVD                                      612
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Fig. 2. Comparison of amino acid sequences of LTA_4 hydrolase deduced for mouse and human. The sequences were aligned by an Intelligenetics (IG) suite (Intelligenetics). Mouse data were obtained from the present study, and human data are from Funk et al. (1987). Short vertical lines represent the aligned identical residues. A one-amino-acid gap in the mouse sequence (His-518) is represented by a hyphen.

3.2. Gene structure of mouse LTA_4 hydrolase

A total of 1×10^6 pfu of a mouse genomic λ Fix II library generated from the 129SvEv strain (Stratagene) was screened using mouse LTA_4 hydrolase cDNA fragments (a mixture of fragments covering the entire coding region) as probes. We identified six positive clones and purified the DNA from them. Restriction mapping and Southern blot analysis were done to identify the different LTA_4 hydrolase sequences containing fragments. The intron–exon organization of the gene was revealed by subcloning the various fragments into pBluescript SK vectors, after which the different exons were sequenced by primer walking. All of the intron–exon boundaries were first verified by sequencing the mouse genomic sequences and then comparing them with mouse cDNA sequences. From this information, a genomic restriction map of 40 kb was constructed showing 20 exons for the mouse LTA_4 hydrolase gene (Fig. 3).

Exon II contained 57 bp of the type II unique sequence and was located approx. 2.8 kbp upstream of exon 1. Exon 1 contained 165 nucleotides of the type I unique 5′ UTR, a common region of 18 bases, the ATG start codon and 52 codons. The last exon (exon 20) consisted of 114 bp of the coding sequence (the last 38 codons) and at least 67 bp of the 3′ UTR. All the intron–exon junctions followed the GT/AG consensus rule (Mount, 1982). The introns varied in length from 200 bp to over 6 kbp. The average length of the exons was 95 bp.

As judged by Southern blot analysis using a battery of restriction endonucleases, mouse LTA_4 hydrolase appeared to be a single-copy gene (data not shown). Our data indicate that the mouse LTA_4 hydrolase and the human gene share the same basic structural organization within the coding region and with respect to the size, number, and splice junctions of their exons. Mouse LTA_4 hydrolase has an additional untranslated exon in its 5′ end.

3.3. Analysis of LTA_4 hydrolase steady-state RNA levels in mouse tissues

We used Northern blot analysis to compare LTA_4 hydrolase expression in a variety of mouse tissues and

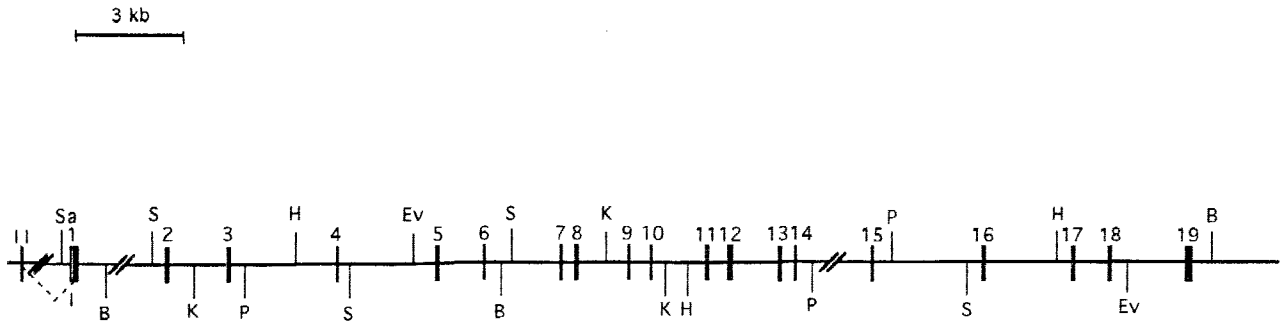

Fig. 3. Genomic intron–exon organization and restriction map of mouse LTA_4 hydrolase gene. The coding exons are denoted by Arabic numerals. The noncoding exons are represented by roman numerals. S, *Sac*II; B, *Bam*HI; H, *Hin*dIII; P, *Pst*I; K, *Kpn*I; RI, *Eco*RI; EV, *Eco*RV. Both the introns and exons are drawn to scale, except for the intron between exon II and exon I, intron 1, and intron 14, all of which are indicated by broken lines. The splicing of the type II exon to the common untranslated exon is indicated by broken, slanted lines.

found that RNA levels were highest in the small intestine, followed by spleen. Appreciable levels of expression were also seen in lung, kidney, and brain. Much lower levels of expression were observed in liver, seminal vesicles, and heart (Fig. 4).

3.4. Evaluation of LTA_4 hydrolase expression in individual cells by in situ hybridization

In situ hybridization to detect LTA_4 hydrolase was performed on a number of tissues using an antisense LTA_4 hydrolase cDNA probe (Fig. 5). In lung, the bronchiolar cells were intensely labeled. Although some interstitial cells were also positive, this labeling was not a prominent feature (Fig. 5A). In spleen, the lymphocytes at the periphery of the follicle were labeled. (Fig. 5B). Positive cells were found in the epithelial lining of the crypts and to a lesser extent, in the villi of the small intestine (Fig. 5C). There was strong staining

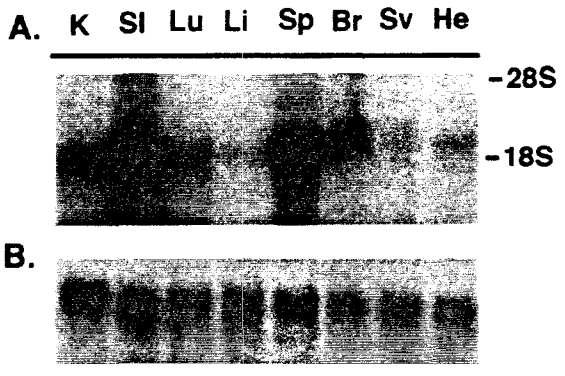

Fig. 4. Northern blot analysis of steady state levels LTA_4 hydrolase expression in different tissues of mice. Top: LTA_4 hydrolase expression in various tissues. K, kidney; SI, small intestine; Lu, lung; Li, liver; Sp, spleen; Br, brain; Sv, seminal vesicles; He, heart. The positions to which 28S and 18S ribosomal RNAs migrated are indicated. The probe used for hybridization is a mouse LTA_4 hydrolase ^{32}P-labeled cDNA probe (see Materials and methods). Bottom: hybridization with a ^{32}P-labeled β-actin probe as a loading control. The blot described in the top panel was stripped and reprobed with β-actin cDNA.

of the epithelium in the seminal vesicles (Fig. 5D) and staining of occasional hepatocytes in liver (Fig. 5E). In kidney, the glomeruli were negative, but the proximal tubules, and to a lesser extent, the collecting ducts were positive (Fig. 5F). Most cells in the adrenal cortex were positive, with strong staining of the zona glomerulosa (Fig. 5G). Myocardial cells showed a pattern of diffuse positivity (Fig. 5H). Thus, our findings on the widespread distribution of LTA_4 hydrolase by in situ hybridization agree with our Northern analysis (Fig. 4) and earlier observations on the tissue distribution of the hydrolase by Izumi et al. (1986).

3.5. Concluding remarks

The widespread expression of LTA_4 hydrolase probably has several causes. First, lymphocytes function at many sites throughout the body. Consequently, there is likely to be a need to regulate them in most tissues. Second, LTA_4 hydrolase is a bifunctional enzyme with an aminopeptidase activity, and aminopeptidase activity is known to be present in many tissues. The bifunctionality of LTA_4 hydrolase will be better understood as the biological substrates for its aminopeptidase activity are discovered. We have no information at present as to whether or not there are two different promoters (represented by type I and type II sequences) (Fig. 1) and whether these promoters function at different sites. It would be interesting, however, to know if these two sequences are in any way related to the separate hydrolase and the aminopeptidase activities.

It is also worth noting that LTA_4 hydrolase activity is highest in the small intestine and spleen. As shown previously by Benajiba and Maroux (1980), the small intestine might be expected to have high levels of aminopeptidase activity. Since we found higher levels of LTA_4 hydrolase expression in the crypt cells, it may be that translation begins in the crypts and the protein moves into the villous in the cytoplasm of maturing cells (Fig. 5C). As for the spleen, it is not surprising

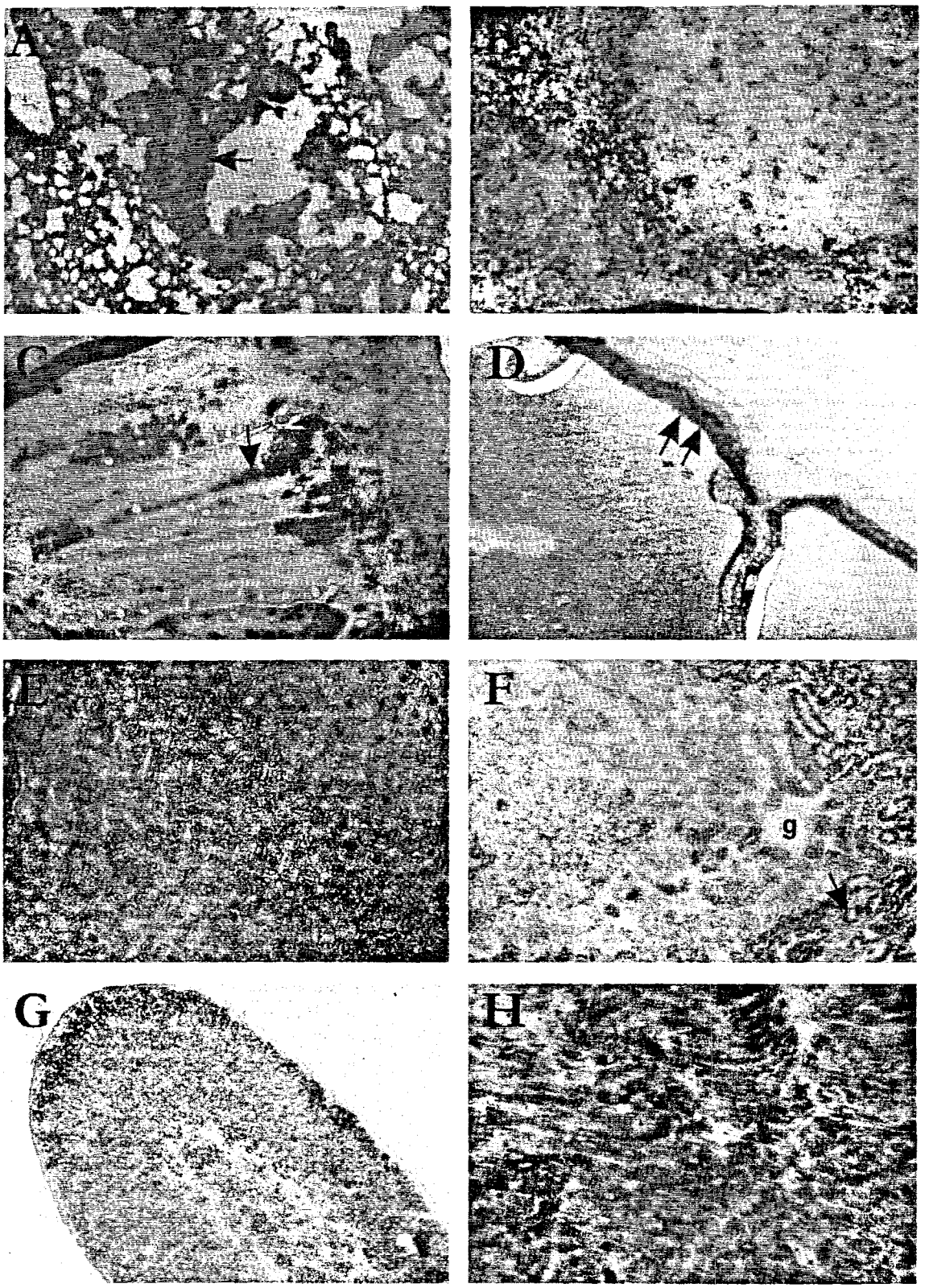

Fig. 5. Demonstration of in situ hybridization for LTA_4 hydrolase in various mouse tissues. In situ hybridization analysis of LTA_4 hydrolase expression in mouse lung (**A**), spleen (**B**), small intestine (**C**), seminal vesicles (**D**), liver (**E**), kidney (**F**), adrenals (**G**), and heart (**H**), respectively. (**A**) Lung section, with labeled bronchiolar cells indicated by solid arrows; 80×. (**B**) Spleen section, with the peripheries of the follicles labeled; 80×. (**C**) Small intestine section, with positive epithelial cells lining the crypt and the villi indicated by arrows; 80×. (**D**) Seminal vesicles, with epithelium intensely stained; 80×. (**E**) Section of the liver showing positivity in occasional hepatocytes. (**F**) Kidney section showing negative glomeruli (g) with positive proximal tubules indicated by arrows; 80×. (**G**) Adrenal gland section showing positive staining of the zona glomerulosa of the adrenal cortex; 50×. (**H**) Heart section showing positive myocardial cells diffuse pattern; 80×.

that we found high levels of LTA_4 hydrolase expression there (Fig. 5B), since LTB_4 is known to play a role in the regulation of lymphoid cells.

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References

Benajiba, A., Maroux, S., 1980. Purification and characterization of an aminopeptidase A from hog intestinal brush-border membrane. Eur. J. Biochem. 107, 381–388.

Boone, D.L., Currie, W.D., Leung, P.C.K., 1993. Arachidonic acid and cell signalling in the ovary and placenta. Prostaglandins Leukotrienes Essent. Fatty Acids 48, 79–87.

Borgeat, P., Samuelsson, B., 1979. Transformation of arachidonic acid by rabbit polymorphonuclear leukocytes: formation of novel dihydroxyeicosatetraenoic acid. J. Biol. Chem. 254, 2643–2646.

Chisholm, D., 1989. A convenient moderate-scale procedure for retaining DNA from bacteriophage lambda. Biotechniques 7, 21–23.

Chomcynki, P., Sacchi, N., 1987. Single step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal. Biochem. 162, 156–159.

Funk, C.D., Radmark, O., Fu, J.Y., Matsumoto, T., Jornvall, H., Shimizu, T., Samuelsson, B., 1987. Molecular cloning and amino acid sequence of leukotriene A_4 hydrolase. Proc. Natl. Acad. Sci. USA 84, 6677–6681.

Goetzl, E.J., Sun, F.F., 1979. Generation of unique monohydroxyeicosatetraenoic acids from arachidonic acid by human neutrophils. J. Exp. Med. 150, 406–411.

Goetzl, E.J., An, S., Smith, W.L., 1995. Specificity of expression and effects of eicosanoid mediators in normal physiology and human diseases. FASEB J. 9, 1051–1058.

Habib, G.M., Barrios, R., Shi, Z.Z., Lieberman, M.W., 1996. Four distinct membrane-bound dipeptidase RNAs are differentially expressed and show discordant regulation with γ-glutamyl transpeptidase. J. Biol. Chem. 271, 16273–16280.

Habib, G.M., Shi, Z.Z., Cuevas, A.A., Guo, Q., Matzuk, M.M., Lieberman, M.W., 1998. Leukotriene D_4 and cystinyl-bis-glycine metabolism in membrane-bound dipeptidase-deficient mice. Proc. Natl. Acad. Sci. USA 95, 4859–4863.

Haggestrom, J.Z., Wetterholm, A., Vallee, B.L., Samuelsson, B., 1990. Leukotriene A_4 hydrolase: an epoxide hydrolase with peptidase activity. Biochem. Biophys. Res. Commun. 173, 431–437.

Henderson Jr., W.R., 1994. The role of leukotrienes in inflammation. Ann. Intern. Med. 121, 684–697.

Izumi, T., Shimizu, T., Seyama, Y., Ohishi, N., Takaku, F., 1986. Tissue distribution of leukotriene A_4 hydrolase activity in guinea pig. Biochem. Biophys. Res. Commun. 135, 139–145.

Lewis, R.A., Austen, K.F., Soberman, R.J., 1990. Leukotrienes and other products of the 5-lipoxygenase pathway: biochemistry and relation to pathobiology in human diseases. New Engl. J. Med. 323, 645–655.

Makita, N., Funk, C.D., Imai, E., Hoover, R.L., Badr, K.F., 1992. Molecular cloning and functional expression of rat leukotriene A_4 hydrolase using polymerase chain reaction. FEBS Lett. 299, 273–277.

Mancini, J.A., Evans, J.F., 1995. Cloning and characterization of human leukotriene A_4 hydrolase gene. Eur. J. Biochem. 231, 56–71.

McGee, J.E., Fitzpatrick, F.A., 1986. Erythrocyte-neutrophil interactions: formation of leukotriene B_4 by transcellular biosynthesis. Proc. Natl. Acad. Sci. USA 83, 1349–1353.

Minami, M., Ohishi, N., Mutoh, H., Izumi, T., Bito, H., Wada, H., Seyama, Y., Toh, H., Shimizu, T., 1990. Leukotriene A_4 hydrolase is a zinc-containing amino peptidase. Biochem. Biophys. Res. Commun. 173, 620–626.

Mount, S.M., 1982. A catalogue of splice junction sequences. Nucleic Acids Res. 10, 459–472.

Orning, L., Gierse, J.K., Duffin, K., Bild, G., Krivi, G., Fitzpatrick, F.A., 1992. Mechanism-based inactivation of leukotriene A_4 hydrolase/aminopeptidase by leukotriene A_4: mass spectrometric and kinetic characterization. J. Biol. Chem. 267, 22733–22739.

Orning, L., Gierse, J.K., Fitzpatrick, F.A., 1994. The bifunctional enzyme leukotriene A_4 hydrolase is an arginine amino peptidase of high efficiency and specificity. J. Biol. Chem. 269, 11269–11273.

Palmer, R.M., Stepney, R.J., Higgs, G.A., Eakins, K.E., 1980. Chemokinetic activities of arachidonic and lipoxygenase products on leukocytes of different species. Prostaglandins 18, 411–418.

Peppelenbosch, M.P., Tertoolen, L.G.J., den Hertog, J., de Laat, S.W., 1992. Epidermal growth factor activates calcium channels by phospholipase A2/5-lipoxygenase-mediated leukotriene C_4 production. Cell 69, 295–303.

Radmark, O., Shimizu, T., Jornvall, H., Samuelsson, B., 1984. Human leukotriene A_4 hydrolase in human leukocytes: Purification and properties. J. Biol. Chem. 259, 12339–12345.

Serhan, C.N., Heggstrom, J.Z., Leslie, C.C., 1996. Lipid mediator networks in cell signaling: update and impact of cytokines. FASEB J. 11, 1147–1158.

Shalati, R.I., Naccache, P.H., Molski, T., Borgeat, P., Goetzl, E.J., 1981. Cellular regulatory role of leukotriene B_4: Its effects on cation homeostasis in rabbit neutrophils. J. Cell Physiol. 108, 401–408.

Stenke, L., Mansour, M., Reizenstein, P., Lindgren, J.A., 1993. Stimulation of human myelopoiesis and leukotriene B_4 and C_4: interactions with granulocyte-macrophage colony-stimulating factor. Blood 81, 352–356.

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