Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 366 (2008) 80–85
BBRC
www.elsevier.com/locate/ybbrc

Leukotriene C_4 synthase promoter driven expression of GFP reveals cell specificity

Tobias Strid, Mats Söderström, Sven Hammarström *

Division of Cell Biology, Department of Clinical and Experimental Medicine, Linköping University, SE-58185 Linköping, Sweden

Received 15 November 2007
Available online 4 December 2007

Abstract

Leukotriene C_4 synthase is a key enzyme in leukotriene biosynthesis. Its gene has been cloned and mapped to mouse chromosome 11. Expression occurs in cells of myeloid origin and also in the choroid plexus, the hypothalamus and the medial eminence of mouse brain. In this study a vector that expresses enhanced green fluorescent protein (eGFP) under the control of the mouse leukotriene C_4 synthase promoter was constructed and used to study promoter activity in different cell lines. Specific eGFP expression was observed in human monocytic leukemia (THP-1) and rat basophilic leukemia (RBL-1) myeloid cells which both express leukotriene C_4 synthase, but not in human embryonic kidney (HEK293/T) epithelial cells which do not express this enzyme. In the myeloid cells, but not in the epithelial cells, we observed that the leukotriene C_4 synthase promoter activity was stimulated by 12-*O*-tetradecanoylphorbol-13-acetate and all-*trans*-retinoic acid. In contrast dimethyl sulfoxide did not affect promoter activity.

Keywords: Leukotriene; Leukotriene C_4 synthase; Expression; GFP; TPA; Promoter; Retinoic acid; DMSO

Cysteinyl leukotrienes are powerful mediators of inflammatory responses and immediate hypersensitivity reactions [1,2]. They are formed from arachidonic acid in activated myeloid cells by sequential reactions catalyzed by 5-lipoxygenase and leukotriene C_4 synthase. The 2.01 kb gene encoding leukotriene C_4 synthase on mouse chromosome 11 comprises five exons with intron/exon boundaries identical to those of the human gene. Promoter-enhancer elements, including binding sites for AP-2 and C/EBP, were recognized in a 1.2 kb 5′-flanking region of the gene [3]. Tissue distribution studies have shown leukotriene C_4 synthase mRNA or protein expression in mouse choroid plexus [4], hypothalamus and brain medial eminence [5].

In this study we report the construction of a plasmid expressing enhanced green fluorescent protein (eGFP) under control of the leukotriene C_4 synthase promoter. Transient transfection of this plasmid into human monocytic leukemia (THP-1), rat basophilic leukemia (RBL-1), and human embryonic kidney (HEK293/T) epithelial cells showed that eGFP was expressed by cells which express leukotriene C_4 synthase (RBL-1 and THP-1) but not by the leukotriene C_4 synthase negative HEK293/T cells. The recombinant vector constructed should be useful for investigations concerning the control of leukotriene C_4 synthase expression *in vitro* and *in vivo*.

Materials and methods

Materials. Restriction enzymes, T4 DNA polymerase, T4 DNA ligase, pcDNA3, competent *E. coli* DH5α, antibiotics, Dulbecco's modified Eagle's medium, and fetal calf serum were obtained from Invitrogen (Paisley Scotland). DE81 anionic exchange paper was purchased from

Abbreviations: AP-2, activating protein 2; C/EBP, CCAAT/enhancer binding protein; eGFP, enhanced green fluorescent protein; HEK293/T, human embryonic kidney 293T cell line; HL-60, human promyelocytic leukemia cell line; LTC_4S, leukotriene C_4 synthase; $pLTC_4S$, leukotriene C_4 synthase promoter; PBS, phosphate buffered saline; RBL-1, rat basophilic leukemia cell line-1; Retinoic acid, all-*trans*-retinoic acid; Sp1, specificity protein 1; Sp3, specificity protein 3; THP-1, human monocytic leukemia cell line; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

* Corresponding author. Fax: +46 13 224149.
E-mail address: sveha@ibk.liu.se (S. Hammarström).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.11.097

Whatman International Ltd (Maidstone, England). A plasmid encoding enhanced green fluorescent protein (p-eGFP-C1) was obtained from Clontech (Mountain View, CA). Plasmid midi purification kit was purchased from Savéen (Malmö, Sweden). Mouse clone RP23-319B15 containing the leukotriene C_4 synthase gene was obtained from BACPAC Resources (Oakland, CA). All other chemicals were from Sigma-Aldrich (St Louis, MO).

Recombinant plasmid. A 1764 bp fragment of the 5′-flanking region of the leukotriene C_4 synthase gene was amplified by PCR from clone BAC RP23-319B15 (see Table 1), and ligated into the EcoRV and XhoI restriction sites of pcDNA3. Another fragment containing intron 2 of the leukotriene C_4 synthase gene was similarly amplified by PCR (Table 1), and ligated into the same plasmid at NheI and XhoI restriction sites. The pLTC$_4$S-intron 2 cassette was verified by sequencing using a MegaBACE 500. eGFP cDNA was amplified by PCR from p-eGFP-C1 (Table 1), and ligated into the XbaI and ApaI sites of an empty pcDNA3 vector. The resulting vector was cleaved by BglII and BamHI to remove its cytomegalovirus promoter and then religated. The insert containing the promoter and intron 2 regions of the leukotriene C_4 synthase gene was excised with EcoRI and XhoI from the pcDNA3 vector and religated into the cut pcDNA3-eGFP vector to generate a recombinant eGFP plasmid driven by the leukotriene C_4 synthase gene promoter (Fig. 1A). The pLTC$_4$S-intron 2-eGFP cassette is readily removable by EcoRI and ApaI digestion.

Cell cultures. Cell cultures were maintained at 37 °C in a humidified 5% CO_2 atmosphere. RBL-1 and THP-1 cells were cultured in RPMI-1640 medium supplemented with 10% (v/v) fetal calf serum, penicillin 100 units/ml, and streptomycin 100 μg/ml. Cell cultures were split 1:10 every third day. HEK293/T cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal calf serum, penicillin 100 units/ml, and streptomycin 100 μg/ml. These cultures were split 1:10 at confluence.

Transfection and other treatments of cells. One hour prior to transfection cells cultured in 6-well plates were washed and 3 ml of serum free medium per well was added. A mixture containing 5 μg recombinant plasmid construct or p-eGFP-C1 control vector, 2 μl 0.1 M polyethyleneimide, and 5 μl 20% (w/v) glucose in a total volume of 22 μl was incubated for 20 min at room temperature and then added to the serum-starved cells. Fetal calf serum was added 3 h after transfection to a final concentration of 10% (v/v) and the cells were incubated for 24 h. At this time final concentrations of 100 nM 12-*O*-tetradecanoylphorbol-13-acetate, 50 μM retinoic acid or 1.5% (v/v) dimethyl sulfoxide were added for another 24 h prior to mounting the cells for microscopic analyses.

Fluorescence microscopy. Cells were washed twice in PBS and fixed with 4% w/v paraformaldehyde. Upon illumination with a 488 nm argon laser eGFP fluorescence was imaged using a Nikon C1 confocal microscope.

Quantitation of fluorescence. The fluorescence in transfected cells was analyzed from images taken on the Nikon microscope, using Volocity 4.1 software (Improvision, Coventry, England). Average fluorescence in individual cells (F) was determined and corrected for background fluorescence in non-transfected cells (B). The corrected fluorescence was

Table 1
Oligonucleotide primers for PCR

Gene sequence	Forward primer	Reverse primer	Product size and position
LTC$_4$S promotor	5′-GCAGATACTGTGCTCGAGATGCG-3′	5′-GGGCAAGCTTGAACAAAGAGACCG-3′	1764 bp (−1230 − +534)
LTC$_4$S intron 2	5′-CTCGAGGAAGAAGATGCCG-3′	5′-CGCTAGCGGTGATCTCTGCAC-3′	237 bp (1068–1304)
eGFP ORF	5′-GCTCTAGAGGTCGCCACCATG-3′	5′-AGTCCGGACTTGTAGGGCCCGTCC-3′	743 bp

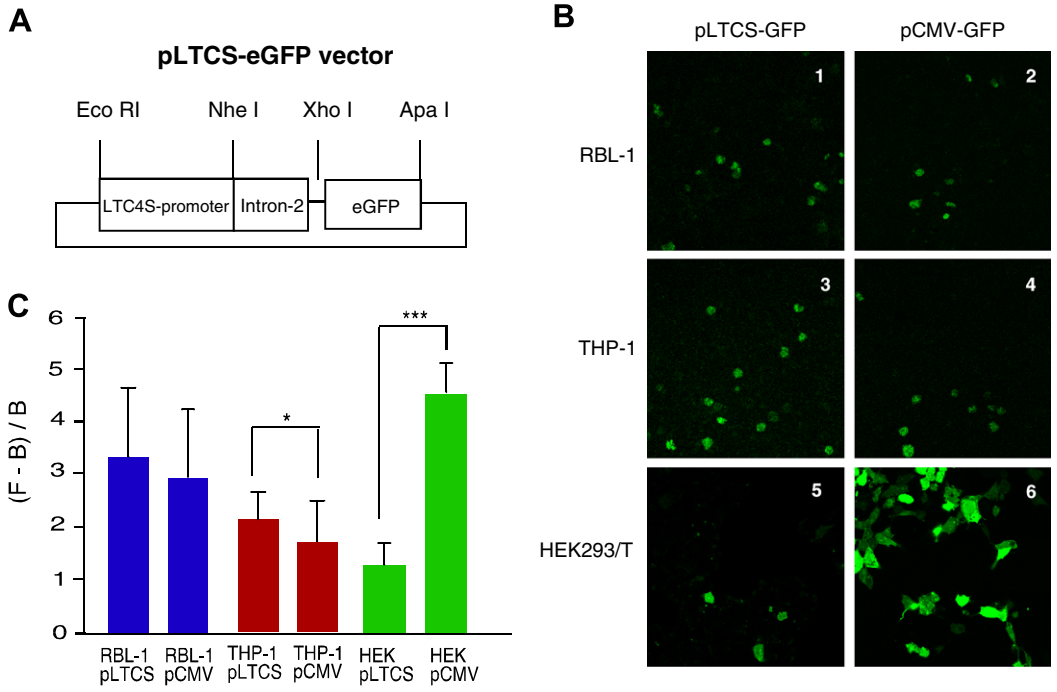

Fig. 1. (A) A vector in which eGFP expression is driven by the promoter and intron 2 of the LTC$_4$ synthase gene was constructed using pcDNA3 as backbone. (B) Fluorescence microscopy of RBL-1 (1 and 2), THP-1 (3 and 4), and HEK293/T (5 and 6) cells transfected with pLTC$_4$S-eGFP or with p-eGFP-C1, a cytomegalovirus promoter (pCMV) driven control plasmid. (C) Quantitative fluorescence measurements on the cells shown in (B). F, average cell fluorescence; B, background fluorescence. The bars show mean values ± SD. Statistical significance is indicated by $^{*}p < 0.05$ or $^{***}p < 0.001$.

divided by the background value [(F-B)/B] to permit comparisons among different images. Mean fluorescence ± SD was calculated for each set of transfections using a non-paired *t*-test.

Results

Cell specific expression of eGFP driven by the leukotriene C_4 synthase promoter

Plasmids expressing eGFP driven by either the leukotriene C_4 synthase (Fig. 1A) or the cytomegalovirus promoter were transfected into RBL-1 and THP-1 cells, which both express leukotriene C_4 synthase and into HEK-293/T cells which do not express leukotriene C_4 synthase. Both RBL-1 and THP-1 cells showed eGFP expression driven by the leukotriene C_4 synthase promoter (Figs. 1B1 and 3) giving fluorescence values 3.4- and 2.1-fold higher than background fluorescence (Fig. 1C). The leukotriene C_4 synthase promoter, in fact, gave higher expression than did the cytomegalovirus promoter (Fig. 1B1–4) in these cells. HEK-293/T cells did not express eGFP under control of the leukotriene C_4 synthase promoter (Fig. 1B5) because their fluorescence did not differ significantly from the background (Fig. 1C). On the other hand HEK-293/T showed high expression of eGFP when driven by the cytomegalovirus promoter (Fig. 1B6). The fluorescence value was 4.6-fold higher than background in these cells compared to the 3- and 1.7-fold increases observed for RBL-1 and THP-1 cells, respectively (Fig. 1C).

Stimulation of leukotriene C_4 synthase promoter activity

The phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA), retinoic acid, and dimethyl sulfoxide stimulate differentiation of myeloid precursor cells and also influence leukotriene C_4 synthase activity. We therefore investigated if these agents would affect leukotriene C_4 synthase promoter activity in RBL-1, THP-1, and HEK-293/T cells (Figs. 2–4). Twenty-four hours after transfection cells were treated with 100 nM TPA, 50 μM retinoic acid or 1.5% (v/v) dimethyl sulfoxide for another 24 h period. Untreated transfected cells were maintained as controls and analogous experiments were also performed using cells transfected with the control vector p-eGFP-C1 that expresses eGFP under control of the cytomegalovirus promoter. The results showed that TPA significantly stimulated leukotriene C_4 synthase promoter driven eGFP expression in

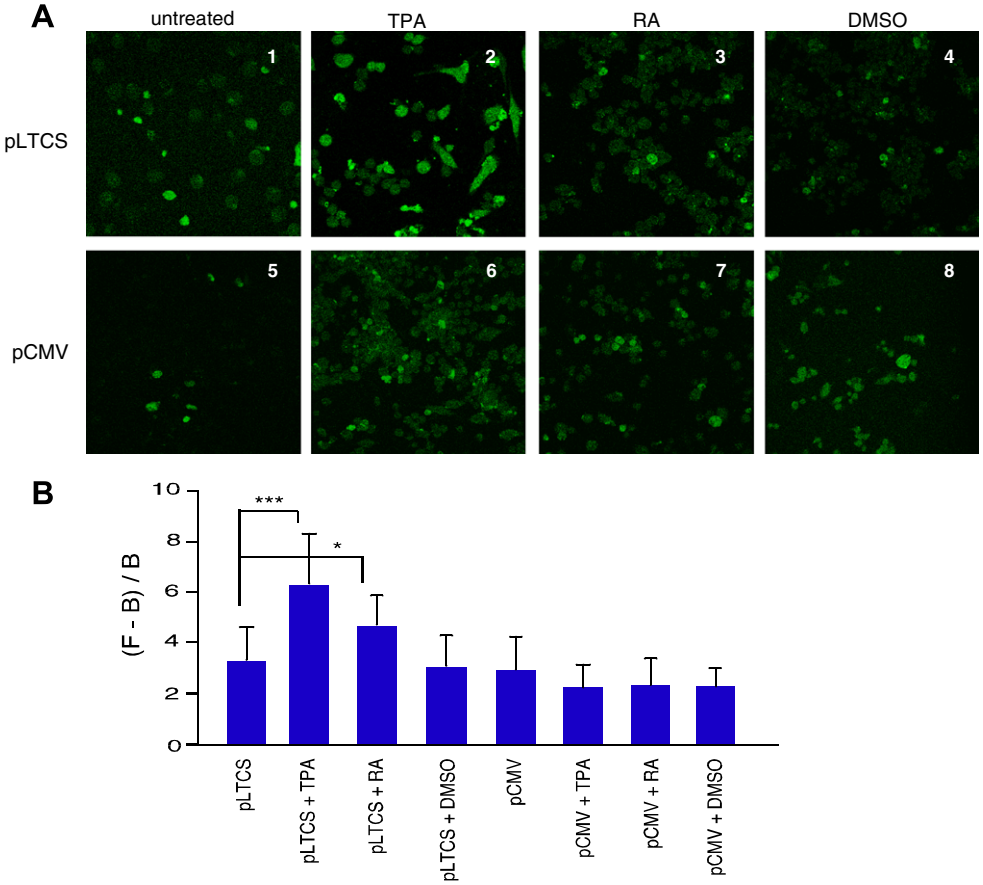

Fig. 2. RBL-1 cells transfected with pLTC$_4$S-eGFP or p-eGFP-C1 were treated for 24 h with TPA (100 nM), retinoic acid (RA, 50 μM) or dimethyl sulfoxide (DMSO, 1.5% v/v) prior to imaging (A) and quantitation of fluorescence (B). TPA and retinoic acid significantly up-regulated LTC$_4$S promoter activity ($^{*}p < 0.05$ or $^{***}p < 0.001$). Dimethyl sulfoxide either had no effect or slightly down-regulated LTC$_4$S promoter activity. Cytomegalovirus promoter (pCMV) driven eGFP expression was unaffected by these agents.

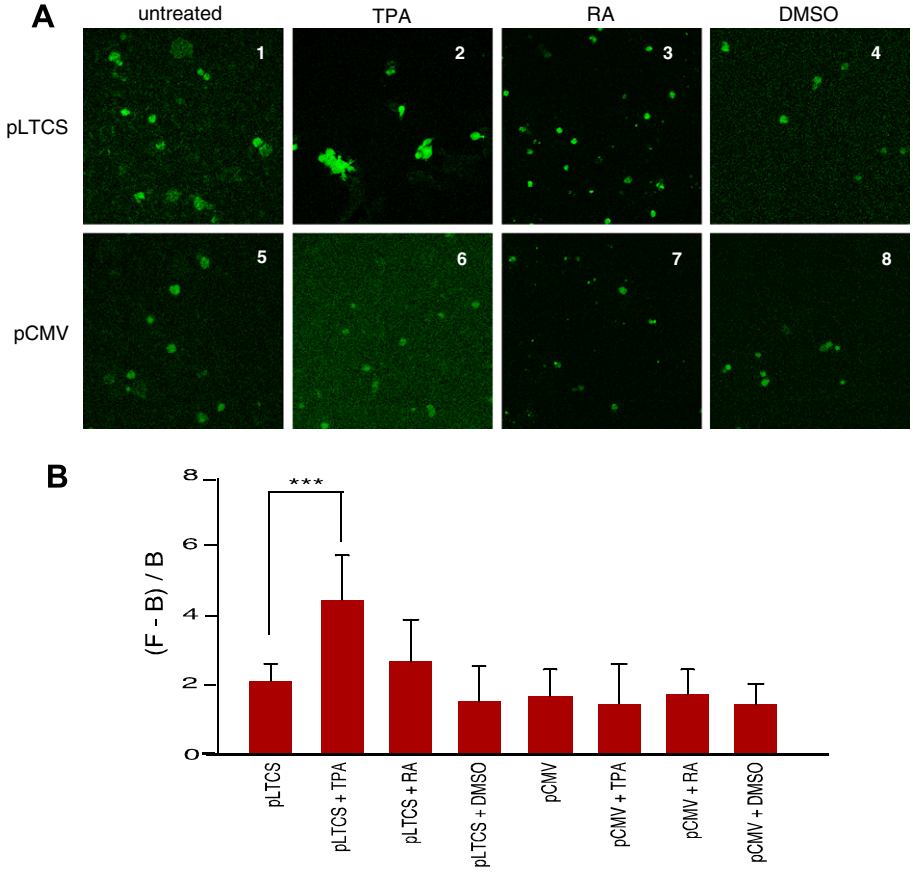

Fig. 3. THP-1 cells transfected with pLTC$_4$S-eGFP or p-eGFP-C1 were treated for 24 h with TPA (100 nM), retinoic acid (RA, 50 μM) or dimethyl sulfoxide (DMSO, 1.5% v/v) prior to imaging (A) and quantitation of fluorescence (B). TPA significantly (***$p < 0.001$) up-regulated LTC$_4$S promoter activity whereas the effect of retinoic acid was not statistically significant. Dimethyl sulfoxide either had no effect or slightly down-regulated LTC$_4$S promoter activity. Cytomegalovirus promoter (pCMV) driven eGFP expression was unaffected by these agents.

both RBL-1 (Fig. 2A2 and B) and THP-1 cells (Fig. 3A2 and B) but not in HEK-293/T cells (Fig. 4A2 and B). The eGFP fluorescence increased from 3.4- and 2.1-fold over background in untreated cells to 6.3- and 4.5-fold over background in RBL-1 and THP-1 cells (Figs. 2B and 3B, respectively). Retinoic acid also stimulated leukotriene C$_4$ synthase promoter activity in RBL-1 cells significantly (Fig. 2A3 and B; (4.7-fold over background) while the smaller increase seen in THP-1 cells (2.7-fold over background) was not significant (Fig. 3A3 and B). When treated with dimethyl sulfoxide a small (but not statistically significant) inhibition of leukotriene C$_4$ synthase promoter driven eGFP expression was observed in RBL-1 (Fig. 2A4 and B) and THP-1 cells (Fig. 3A4 and B). In contrast, no measurable effect was observed on cytomegalovirus promoter driven expression (Figs. 2A6–8 and B; 3A6–8 and 3B; 4A6–8 and B).

Discussion

Leukotriene C$_4$ synthase mRNA and protein expression in brain have been shown to be restricted using *in situ* hybridization and immunohistochemistry techniques [4,5]. To investigate this further a plasmid was constructed where the expression of eGFP is driven by the leukotriene C$_4$ synthase promoter. The plasmid was transfected into two myeloid cell lines, RBL-1 and THP-1, known to express leukotriene C$_4$ synthase and also into an epithelial cell line which does not express the enzyme. As expected, eGFP was formed by the cells which express leukotriene C$_4$ synthase but not by the cells which do not express this enzyme. In a previous study TPA was found to stimulate leukotriene C$_4$ synthase activity in human erythroleukemia cells whereas dimethyl sulfoxide inhibited the activity and retinoic acid had no effect [6]. The stimulatory effect of TPA was confirmed in this study suggesting that it occurs at the promoter level. It is unlikely that it was indirectly caused by cell differentiation because the differentiating agents used had different effects on leukotriene C$_4$ synthase promoter activity. The lack of effect of the various treatments in HEK-293/T control cells and in cells transfected with control plasmid further supported this notion. Several potential regulatory elements were identified in the 5′-flanking region of the leukotriene C$_4$ synthase gene [3]. Of these elements, Sp1 and Sp3, were found to increase transcription of the gene [7]. Sp1 and a Krüppel-like transcription factor have been suggested to determine cell-specific leukotriene C$_4$ synthase transcription [8]. The

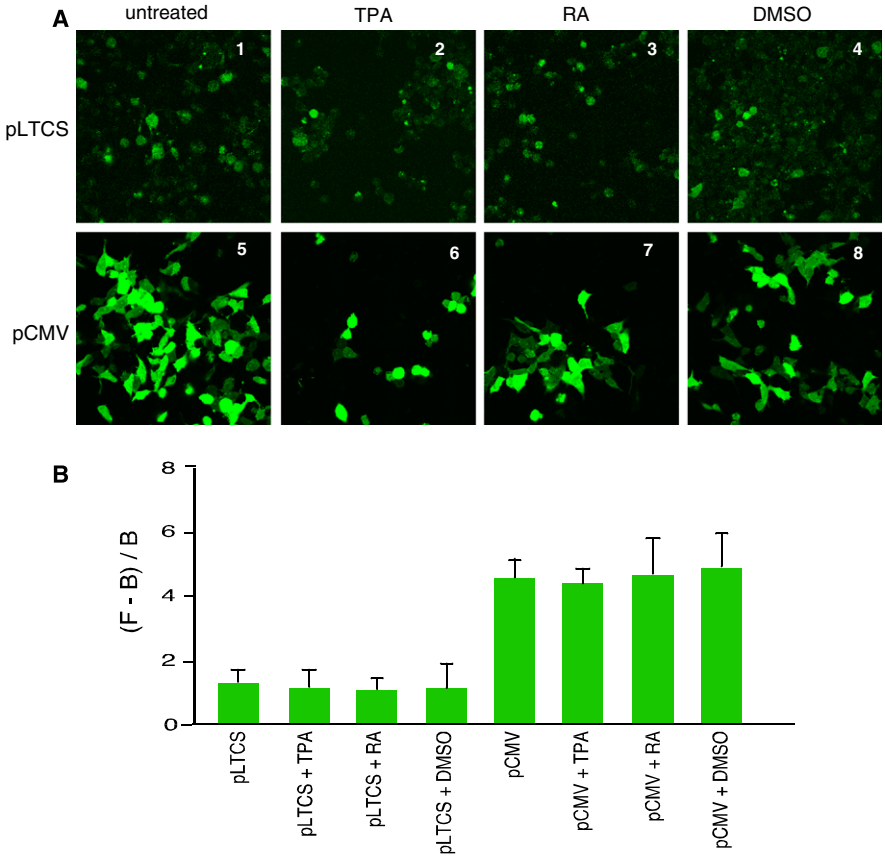

Fig. 4. HEK-293/T cells transfected with pLTC$_4$S-eGFP or p-eGFP-C1 were treated for 24 h with TPA (100 nM), retinoic acid (RA, 50 μM) or dimethyl sulfoxide (DMSO, 1.5% v/v) prior to imaging (A) and quantitation of fluorescence (B). Neither TPA, retinoic acid nor dimethyl sulfoxide affected pLTC$_4$S or cytomegalovirus promoter (pCMV) driven eGFP expression.

mechanism by which TPA and retinoic acid treatment affect the activity of the leukotriene C_4 synthase promoter is not known: TPA induced leukotriene C_4 synthesis in mouse skin [9] as well as in cell lines [6,10]. Others have reported that TPA decreased leukotriene C_4 production in HL-60 cells and that this effect was blocked by PKC specific inhibitors [11]. Furthermore, retinoic acid up-regulated leukotriene C_4 synthase in rat basophilic leukemia cells [12,13]. Thus it appears that leukotriene C_4 synthase gene expression is differentially regulated depending on the cellular environment, in agreement with the results presented here.

Acknowledgments

Rat basophilic leukemia cells (RBL-1) was a kind gift from M. Hansson and A.M. Persson, Lund University. This work was supported by grants from the Swedish Research Council (31X-05914) and Östergötland County Council.

References

[1] B. Samuelsson, S.E. Dahlen, J.-Å. Lindgren, C.A. Rouzer, C.N. Serhan, Leukotrienes and lipoxins: structures, biosynthesis, and biological effects, Science 237 (1987) 1171–1176.

[2] B. Samuelsson, S. Hammarström, Leukotrienes: a novel group of biologically active compounds, Vitam. Horm. 39 (1982) 1–30.

[3] J.F. Penrose, M.H. Baldasaro, M. Webster, K. Xu, K.F. Austen, B.K. Lam, Molecular cloning of the gene for mouse leukotriene C_4 synthase, Eur. J. Biochem. 248 (1997) 807–813.

[4] M. Söderström, D. Engblom, A. Blomqvist, S. Hammarström, Expression of leukotriene C_4 synthase mRNA by the choroid plexus in mouse brain suggests novel neurohormone functions of cysteinyl leukotrienes, Biochem. Biophys. Res. Commun. 307 (2003) 987–990.

[5] A. Shimada, M. Satoh, Y. Chiba, Y. Saitoh, N. Kawamura, H. Keino, M. Hosokawa, T. Shimizu, Highly selective localization of leukotriene C_4 synthase in hypothalamic and extrahypothalamic vasopressin systems of mouse brain, Neuroscience 131 (2005) 683–689.

[6] M. Söderström, A. Bolling, S. Hammarström, Induction of leukotriene C_4 synthase activity in differentiating human erythroleukemia cells, Biochem. Biophys. Res. Commun. 189 (1992) 1043–1049.

[7] K.J. Serio, C.R. Hodulik, T.D. Bigby, Sp1 and Sp3 function as key regulators of leukotriene C_4 synthase gene expression in the monocyte-like cell line, THP-1, Am. J. Respir. Cell Mol. Biol. 23 (2000) 234–240.

[8] J.L. Zhao, K.F. Austen, B.K. Lam, Cell-specific transcription of leukotriene C_4 synthase involves a Krüppel-like transcription factor and Sp1, J. Biol. Chem. 275 (2000) 8903–8910.

[9] Y. Nakamura, M. Kozuka, K. Naniwa, S. Takabayashi, K. Torikai, R. Hayashi, T. Sato, H. Ohigashi, T. Osawa, Arachidonic acid cascade inhibitors modulate phorbol ester-induced oxidative stress in female ICR mouse skin: differential roles of 5-lipoxygenase and cyclooxygenase-2 in leukocyte infiltration and activation, Free Radic. Biol. Med. 35 (2003) 997–1007.

[10] R. Akasaka, R. Teshima, H. Ikebuchi, J. Sawada, Effects of three different Ca^{2+}-ATPase inhibitors on Ca^{2+} response and leukotriene release in RBL-2H3 cells, Inflamm. Res. 45 (1996) 583–589.

[11] A. Ali, A.W. Ford-Hutchinson, D.W. Nicholson, Activation of protein kinase C down-regulates leukotriene C_4 synthase activity and attenuates cysteinyl leukotriene production in an eosinophilic substrain of HL-60 cells, J. Immunol. 153 (1994) 776–788.

[12] M. Abe, K. Shibata, S. Saruwatar, S. Soeda, H. Shimeno, T. Katsuragi, cDNA cloning and expression of rat leukotriene C_4 synthase: elevated expression in rat basophilic leukemia-1 cells after treatment with retinoic acid, Prostaglandins Leukot. Essent. Fatty Acids 67 (2002) 319–326.

[13] M. Abe, K. Shibata, H. Urata, N. Sakata, T. Katsuragi, Induction of leukotriene C_4 synthase after the differentiation of rat basophilic leukemia cells with retinoic acid and a low dose of actinomycin D and its suppression with methylprednisolone, J. Cell Physiol. 196 (2003) 154–164.