REGULATION OF LEUKOTRIENE C₄ SYNTHASE GENE EXPRESSION BY SP₁ AND SP₃ IN MONONUCLEAR PHAGOCYTES

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INTRODUCTION

A large body of experimental evidence has implicated the cysteinyl leukotrienes in a variety of inflammatory responses¹². Leukotriene C₄ (LTC₄) synthase (M_r of 16,568; EC#2.5.1.37) is a selective, membrane-bound glutathione-S-transferase that catalyzes the conversion of LTA₄ to LTC₄. Although LTC₄ synthase enzymatic activity is found in various cell types, mRNA for LTC₄ synthase is present only in eosinophils, basophils, mast cells, and cells of monocyte/macrophage lineage. Previous studies suggest that LTC₄ synthase expression is increased in the bronchial mucosa of patients with aspirin-sensitive asthma³. The gene for LTC₄ synthase has been cloned, sequenced, and mapped to the distal region of chromosome 5⁴,⁵. The LTC₄ synthase 5' flanking region lacks a TATA box but has known consensus sites for ets, AP-1, AP-2, and Sp¹⁴,⁵. Previous work from our laboratory indicates that LTC₄ synthase gene expression is upregulated by transforming growth factor-β (TGF-β) through a transcriptional mechanism in the monocyte-like cell line, THP-1⁶. The purpose of this study was to begin to investigate the molecular mechanisms of regulation of transcription of the LTC₄ synthase gene in mononuclear phagocytes.
MATERIALS AND METHODS

Cell Culture
THP-1 were grown at 37 °C with 5% CO2 in RPMI 1640 medium supplemented with 10% heat-treated fetal calf serum (FCS). HeLa cells were grown at 37 °C with 5% CO2 in MEM supplemented with 10% FCS. Drosophila SL2 cells were grown at 25 °C in Schneider's Drosophila medium supplemented with 10% FCS.

Screening of THP-1 and HeLa Cells for LTC4 Synthase mRNA
Total RNA was extracted from THP-1 and HeLa cells and was subjected to reverse transcriptase-polymerase chain reaction (RT-PCR). A polymerase chain reaction (PCR) reaction was then performed to screen for LTC4 synthase cDNA, as previously described. The expected PCR product of 103 bp was electrophoresed through an agarose gel and visualized by ethidium bromide staining.

Construction of the Luciferase Promoter-Reporter Constructs
A 1.3 kb fragment of the LTC4 synthase promoter (starting at +119 relative to the transcription start site) was prepared by PCR amplification from a human genomic DNA clone by a previously described technique. Successive 5'-deletions of the LTC4 synthase promoter were accomplished utilizing PCR reactions performed on this plasmid. The PCR products were isolated and subsequently ligated into the pGL3 Basic plasmid (Promega; Madison, WI). Constructs with site-directed mutations were created by introducing a two base pair substitution (AA for CG at -38 and -39 bp) within the Sp1 sites of the wild-type promoter-reporter constructs.

Cell Transfection and Reporter Gene Assays
THP-1 and HeLa cells were transfected with promoter-constructs and a pCMV-β-galactosidase plasmid. Drosophila SL2 cells were transfected with promoter-reporter constructs and Drosophila actin promoter-driven expression vectors for Sp1 and Sp3. Following incubation, the cells were lysed and assayed for luciferase and β-galactosidase activity.

Electrophoretic Mobility Shift Assay (EMSA)
Nuclear extracts were prepared from THP-1 and HeLa cells. EMSAs were performed with extracts or purified Sp1 utilizing a double-stranded probe from -56 to -17 bp of the promoter. The probe was incubated and monoclonal anti-Sp1 and/or anti-Sp3 antibodies were subsequently added. The samples were incubated and subjected to electrophoresis on a 4% non-denaturing polyacrylamide gel. The gel was dried and exposed to autoradiographic film.

RESULTS AND DISCUSSION

Screening of THP-1 and HeLa Cells for LTC4 Synthase mRNA