

Cysteinyl Leukotrienes Induce Monocyte Chemoattractant Protein-1 in Human Monocyte/Macrophages via Mitogen-Activated Protein Kinase and Nuclear Factor- κ B Pathways

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Key Words

Cysteinyl leukotriene • Monocyte chemoattractant protein-1 • Mitogen-activated protein kinase • Nuclear factor- κ B • Monocyte/macrophages

Abstract

Background: We have previously demonstrated that cysteinyl leukotriene (CysLT) induced monocyte chemoattractant protein-1 (MCP-1) production in monocytes/macrophages. The intracellular signal transduction pathway of MCP-1 production induced by CysLT in human monocytes/macrophages is unclear. **Methods:** The activation of mitogen-activated protein kinase (MAPK), including extracellular signal-regulated kinases 1/2 (ERK1/2), c-Jun N-terminal kinase (JNK) and p38 MAPK by phosphorylation, and nuclear factor- κ B (NF- κ B) by leukotriene (LT) D₄ and LTC₄ was determined in THP-1 cells, a human monocytic leukemia cell line, and peripheral blood CD14⁺ monocytes/macrophages. We examined the inhibitory effects of inhibitors of ERK1/2, JNK, p38 MAPK and NF- κ B and pranlukast as a CysLT1 receptor antagonist on induction of MCP-1 production by LTD₄ and LTC₄. **Results:** LTD₄ and LTC₄ induced significant phosphorylations of ERK1/2 and JNK, but not p38 MAPK, in THP-1 cells

and peripheral blood CD14⁺ monocytes/macrophages. Pretreatment with the ERK1/2 inhibitor PD98059 and JNK inhibitor SP600125 attenuated MCP-1 production by CysLTs. NF- κ B activation was induced by addition of LTD₄ and LTC₄. Pretreatment with the NF- κ B inhibitors caffeic acid phenylethyl ester and MG-132 inhibited MCP-1 production by CysLTs. Pranlukast inhibited phosphorylation of ERK1/2 and JNK, NF- κ B activation, and the MCP-1 production induced by CysLTs. **Conclusion:** CysLTs induce MCP-1 and this induction is mediated by ERK1/2 and JNK in MAPK, and NF- κ B pathways via the CysLT1 receptor, for the most part, in human monocytes/macrophages.

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Introduction

Leukotriene D₄ (LTD₄), one of the cysteinyl leukotrienes (CysLT), induces contraction of the tracheal muscle and has potent effects on leukocyte trafficking, airway mucus secretion and collagen synthesis in bronchial asthma [1–3]. Normal peripheral blood leukocytes, such as basophils, eosinophils, B lymphocytes and monocytes/macrophages, have a CysLT1 receptor [4]. Monocyte che-

moattractant protein-1 (MCP-1), a member of the C-C chemokine β -subfamily, chemoattracts monocytes and T lymphocytes and is produced by various cells such as monocytes/macrophages, lymphocytes and airway epithelial cells [5, 6]. MCP-1 has multiple functions, such as mast cell aggregation and histamine release in mast cells [7, 8]. MCP-1 causes not only the migration of monocytes, but also of eosinophils and basophils, and induces basophil histamine release [5]. It is likely that MCP-1 mediates the pathogenesis of bronchial asthma. We have already demonstrated that CysLTs induce MCP-1 production in human monocytes/macrophages [9]. However, intracellular signal transduction pathways of MCP-1 production induced by CysLTs in monocytes/macrophages is poorly understood.

Mitogen-activated protein kinase (MAPK) belongs to a group of serine threonine kinases [10]. They are major players in mediating a variety of signals for cellular functions, including cell mitosis, apoptosis and activation of transcription of some inflammatory cytokines [11]. Mammalian cells have 3 major MAPK subfamilies: extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 MAPK [12].

Nuclear factor- κ B (NF- κ B) is a ubiquitous transcription factor for genes that encode proinflammatory cytokines, chemokines and adhesion molecules that mediate inflammation [13, 14].

We investigated whether or not MAPK and NF- κ B pathways mediate MCP-1 production induced by LTD₄ and LTC₄ in THP-1 cells, a human monocytic leukemia cell line, and human peripheral blood CD14⁺ monocytes/macrophages.

Methods

Cell Culture and Stimulation Conditions

THP-1 cells [15], a human monocytic leukemia cell line that has a CysLT1 receptor [16], obtained from the American Type Culture Collection, were maintained at 37°C under humidified 5% CO₂ as stationary cultures. The cells were grown in RPMI 1640 containing 10% FBS, 100 U/ml penicillin and 100 μ g/ml streptomycin.

Peripheral blood mononuclear cells were obtained from heparinized blood from 8 healthy medication-free volunteers, with informed consent, by Histopaque 1077 (Sigma Chemical Co., St. Louis, Mo., USA) gradient centrifugation and washing. Purification of individual cell subpopulations was achieved with a high magnetic gradient Mini MACS purification system (Miltenyi, Sunnyvale, Calif., USA). CD14⁺ monocytes/macrophages were isolated by depletion of nonmonocytes (negative selection) with a Monocyte Isolation Kit II (Miltenyi). The purity of the isolated cells was determined using the respective fluorescein isothio-

cyanate-conjugated monoclonal antibodies (Becton-Dickinson Biosciences, San Diego, Calif., USA) and flow-cytometric analysis (FACSscan; Becton-Dickinson Biosciences).

Cells were exposed to LTD₄ or LTC₄ (Cayman Chemical, Ann Arbor, Mich., USA). Some samples were pretreated with pranlukast, a CysLT1 receptor antagonist, provided by ONO Pharmaceutical Co. (Osaka, Japan), ERK1/2 inhibitor PD98059, JNK inhibitor SP600125, p38 MAPK inhibitor SB 203580, purchased from Calbiochem Corp. (San Diego, Calif., USA), and the NF- κ B inhibitor caffeic acid phenylethyl ester (CAPE) and MG-132 (Calbiochem) for 30 min before the addition of LTD₄ or LTC₄.

Quantification of Phosphorylated and Total ERK1/2, JNK and p38 MAPK

The levels of phosphorylated and total ERK1/2, JNK and p38 MAPK were quantified by cell-based ELISA using fast activated cell-based ELISA kits (Active Motif, Carlsbad, Calif., USA) according to the manufacturer's instructions. Cells (2×10^5 cells/well) were attached to 96-well culture plates that were coated with 10 μ g/ml poly-L-lysine (Sigma) for 30 min at 37°C, and incubated overnight at 37°C under humidified 5% CO₂. Cells on culture plates were exposed to 10^{-6} to 10^{-8} M LTD₄ or 10^{-6} M LTC₄ for 10 or 30 min. The cells were fixed with 8% formaldehyde solution, and incubated with specific antiphosphorylated or total anti-MAPK antibodies followed by incubation with a secondary horseradish peroxidase-conjugated antibody. The levels of phosphorylated/total MAPKs were quantified using colorimetric readout. The measured OD₄₅₀ readings in the quantification of the phosphorylated/total MAPKs were corrected for cell number by dividing the OD₄₅₀ reading for a given well by the crystal violet OD₅₉₅ reading. The data were expressed as the ratio of phosphorylated/total MAPKs.

Nuclear Extracts and Determination of NF- κ B Activation

Nuclear extracts were harvested from THP-1 cells and peripheral blood CD14⁺ monocytes/macrophages by Nuclear Extract kits (Active Motif) according to the manufacturer's instructions. The protein concentrations of nuclear extracts were determined using Coomassie Plus Protein Assay Reagent (Pierce, Rockford, Ill., USA). The levels of NF- κ B were determined with an activated NF- κ B ELISA kit (Active Motif). An oligonucleotide containing the NF- κ B consensus site (5'-GGGACTTTC-3') was adsorbed to polystyrene microwells. The activated NF- κ B contained in nuclear extracts binds specifically to this oligonucleotide, and the NF- κ B complex bound to the oligonucleotide can be detected using an anti-NF- κ B p65 antibody. Following this, a secondary antibody conjugated to horseradish peroxidase was added. The absorbance was measured at 450 nm by spectrophotometry.

Determination of MCP-1 Concentrations

The concentration of MCP-1 in the supernatant was determined with sandwich-type ELISA kit (R&D Systems, Minneapolis, Minn., USA). The detection limit for MCP-1 was 5 pg/ml.

Statistical Analysis

The data were presented as means $\pm$ SD. Statistical analysis was performed with the Wilcoxon matched paired test, with a *p* value of less than 0.05 being taken as significant. Analyses and calculations were performed using SPSS 12.0 (SPSS Inc., Chicago, Ill., USA).

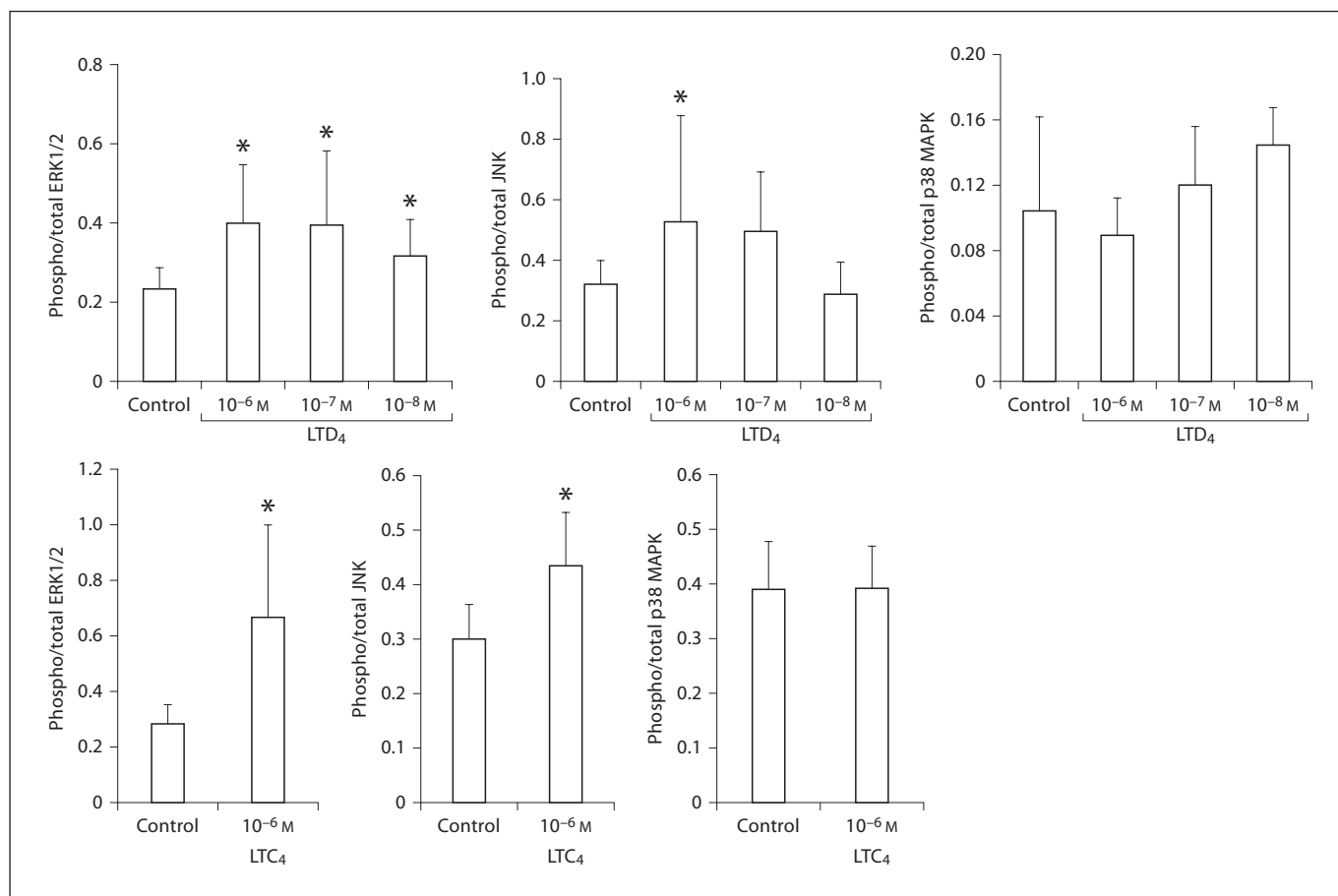


Fig. 1. The ratio of phosphorylated/total ERK1/2, JNK and p38 MAPK stimulated with LTD₄ and LTC₄ for 10 min in THP-1 cells, as measured by cell-based ELISA. * $p < 0.05$, compared to cells cultured in medium including 0.2% DMSO. Data are expressed as means $\pm$ SD of 8 experiments.

Results

Figure 1 demonstrates the ratio of phosphorylated/total for 3 major subfamilies of MAPKs stimulated with LTD₄ and LTC₄ for 10 min in THP-1 cells. Significant phosphorylation of ERK1/2 was induced by stimulation with 10⁻⁶, 10⁻⁷ and 10⁻⁸ M LTD₄ and 10⁻⁶ M LTC₄ (all $p < 0.05$), and that of JNK by stimulation with 10⁻⁶ M LTD₄ and LTC₄ (both $p < 0.05$), with no significant phosphorylation of p38 MAPK by LTD₄ or LTC₄. Stimulation with LTD₄ or LTC₄ for 30 min did not induce significant phosphorylation of ERK1/2, JNK or p38 MAPK (data not shown). LTD₄ or LTC₄ did not affect total ERK1/2, JNK or p38 MAPK (data not shown). Figure 2 demonstrates the ratio of phosphorylated/total for 3 major subfamilies of MAPKs stimulated with LTD₄ for 10 min in peripheral blood CD14⁺ monocytes/macrophages. Significant

phosphorylations of ERK1/2 and JNK were induced by 10⁻⁶ and 10⁻⁷ M LTD₄ (all $p < 0.05$), with no significant phosphorylation of p38 MAPK. Pranlukast completely inhibited phosphorylations of ERK1/2 and JNK induced by 10⁻⁶ M LTD₄ in THP-1 cells (fig. 3).

Figure 4 illustrates the effects of MAPK inhibitors and pranlukast on MCP-1 production induced by LTD₄ in THP-1 cells. Pretreatment with ERK1/2 inhibitor PD98059 and JNK inhibitor SP600125 reduced MCP-1 production induced by LTD₄ (both $p < 0.05$). In contrast, pretreatment with p38 MAPK inhibitor SB203580 enhanced MCP-1 production in the presence of LTD₄ ($p < 0.05$). Pranlukast completely inhibited MCP-1 production induced by LTD₄ ($p < 0.01$). Figure 5 shows the effects of MAPK inhibitors and pranlukast on MCP-1 production induced by LTC₄ in THP-1 cells. Pretreatment with ERK1/2 inhibitor PD98059 and JNK inhibitor

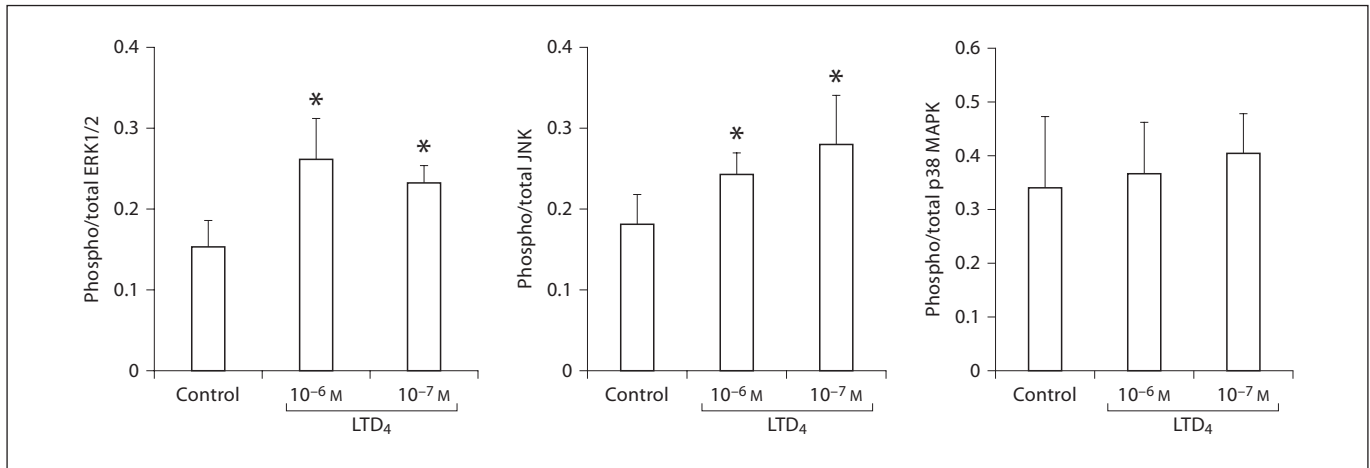
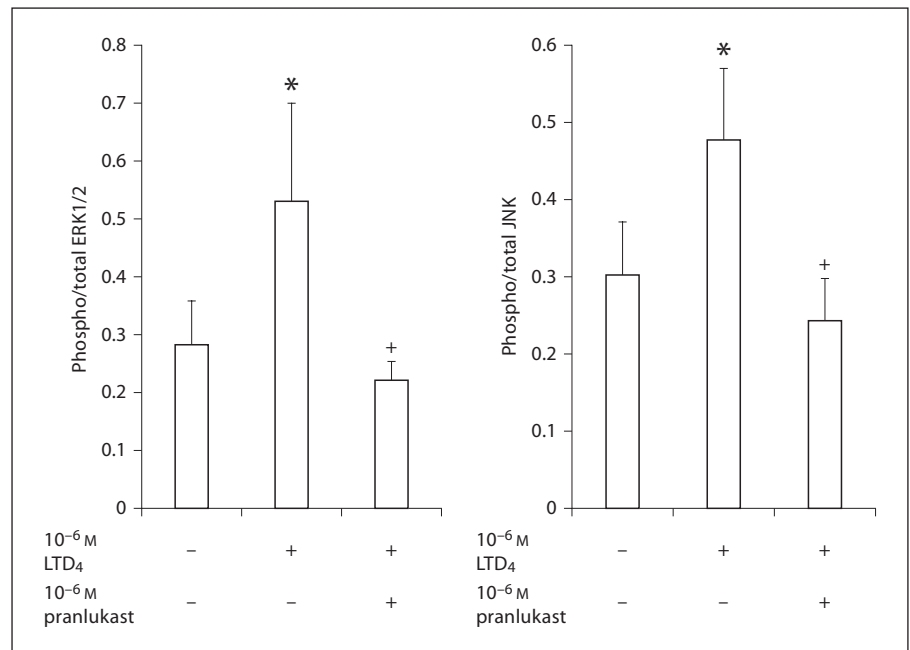


Fig. 2. The ratio of phosphorylated/total ERK1/2, JNK and p38 MAPK stimulated with LTD₄ for 10 min in peripheral blood CD14⁺ monocytes/macrophages, as measured by cell-based ELISA. * $p < 0.05$, compared to cells cultured in medium including 0.2% DMSO. Data are expressed as means $\pm$ SD of 8 experiments.

Fig. 3. Inhibitory effect of pranlukast on phosphorylation of ERK1/2 and JNK induced by LTD₄ for 10 min in THP-1 cells, as measured by cell-based ELISA. Cells were pretreated with 10⁻⁶ M pranlukast for 30 min before LTD₄ treatment. * $p < 0.05$, compared to cells cultured in medium including 0.2% DMSO. + $p < 0.05$, compared to cells treated with LTD₄ only. Data are expressed as means $\pm$ SD of 8 experiments.



SP600125 reduced MCP-1 production induced by LTC₄ (both $p < 0.05$). In contrast, pretreatment with p38 MAPK inhibitor SB203580 enhanced MCP-1 production in the presence of LTC₄ ($p < 0.05$). Pranlukast completely inhibited MCP-1 production induced by LTC₄ ($p < 0.01$).

NF- κ B activation induced by LTD₄ and LTC₄, and the inhibitory effects of pranlukast in THP-1 cells are shown in figure 6. NF- κ B activation was significantly induced

by stimulation with LTD₄ and LTC₄ for 10 min ($p < 0.01$ and $p < 0.05$, respectively), and pretreatment with pranlukast significantly inhibited NF- κ B activation ($p < 0.01$ and $p < 0.05$, respectively). Figure 7 demonstrates that LTD₄ significantly induced NF- κ B activation ($p < 0.05$) and pretreatment with pranlukast significantly inhibited NF- κ B activation ($p < 0.05$) in peripheral blood CD14⁺ monocytes/macrophages. NF- κ B inhibitors CAPE and

Fig. 4. Inhibitory effect of MAPK inhibitors and pranlukast on MCP-1 release in culture fluid, as measured by ELISA in THP-1 cells stimulated with 10^{-6} M LTD₄ for 4 h. Cells were pretreated with 10^{-6} M ERK1/2 inhibitor PD98059, JNK inhibitor SP600125, p38 inhibitor SB203580 and pranlukast for 30 min before LTD₄ treatment. Data (n = 8) are presented as means $\pm$ SD. ** p < 0.01, compared to culture fluid of cells in medium including 0.2% DMSO. ++ p < 0.01 and + p < 0.05, compared to culture fluid of cells treated with LTD₄ only.

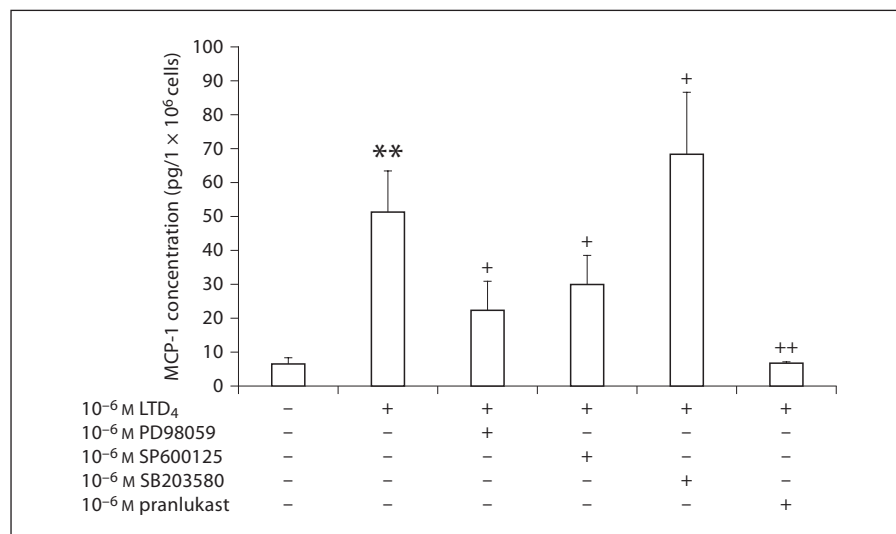
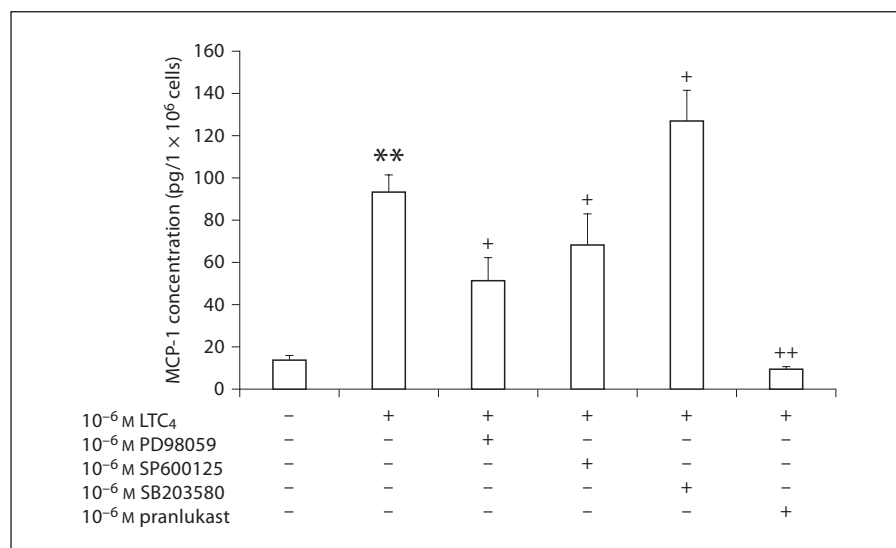


Fig. 5. Inhibitory effect of MAPK inhibitors and pranlukast on MCP-1 release in culture fluid, as measured by ELISA in THP-1 cells stimulated with 10^{-6} M LTC₄ for 4 h. Cells were pretreated with 10^{-6} M ERK1/2 inhibitor PD98059, JNK inhibitor SP600125, p38 inhibitor SB203580 and pranlukast for 30 min before LTC₄ treatment. Data (n = 8) are presented as mean $\pm$ SD. ** p < 0.01, compared to culture fluid of cells in medium including 0.2% DMSO. ++ p < 0.01 and + p < 0.05, compared to culture fluid of cells treated with LTC₄ only.



MG-132 significantly inhibited MCP-1 production induced by LTD₄ and LTC₄ in THP-1 cells (all p < 0.05), as shown in figure 8.

Discussion

Bronchial asthma is characterized by chronic airway inflammation [17]. In bronchial asthma, MCP-1 plays an important role in lung allergic inflammation, leukocyte infiltration and bronchial hyperresponsiveness [5]. Alveolar macrophages are the most abundant cells, not only in the alveoli and distal air spaces but also in the conducting airways [18]. Activated alveolar macrophages may

participate in the inflammatory events associated with allergic disease of the lower airways, including release of cytokines, chemokines, arachidonic acid metabolites, products of activated O₂, and by direct interaction with T lymphocytes [19–23]. These studies suggested that MCP-1 and macrophages play important roles in the pathogenesis of bronchial asthma. CysLTs modulate the pathogenesis of bronchial asthma [24]. We have already reported that CysLTs induced MCP-1 in human monocytes/macrophages [9]. We investigated MAPK pathways in MCP-1 production induced by LTD₄ and LTC₄ in human monocytes/macrophages in the present study. Regarding MAPK pathways, the ERK-signaling pathway in antigen-presenting cells may promote the initial commit-

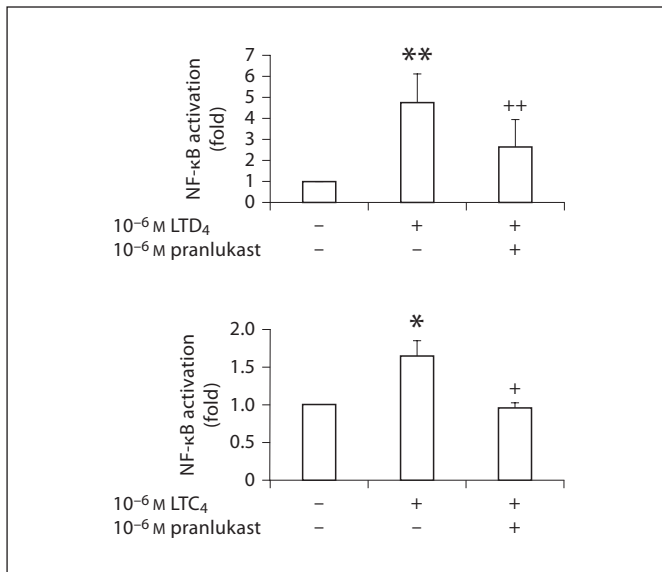


Fig. 6. NF-κB activation induced by LTD₄ or LTC₄ stimulation of THP-1 cells, as measured by ELISA. Cells are treated with LTD₄ or LTC₄ for 10 min. Pranlukast was added 30 min before LTD₄ or LTC₄ stimulation. The data (n = 8) are expressed as the ratio of stimulated cells to cells cultured in medium including 0.2% DMSO, and presented as means ± SD. ** p < 0.01 and * p < 0.05, compared to cells cultured in medium including 0.2% DMSO. ++ p < 0.01 and + p < 0.05, compared to cells treated with LTD₄ or LTC₄.

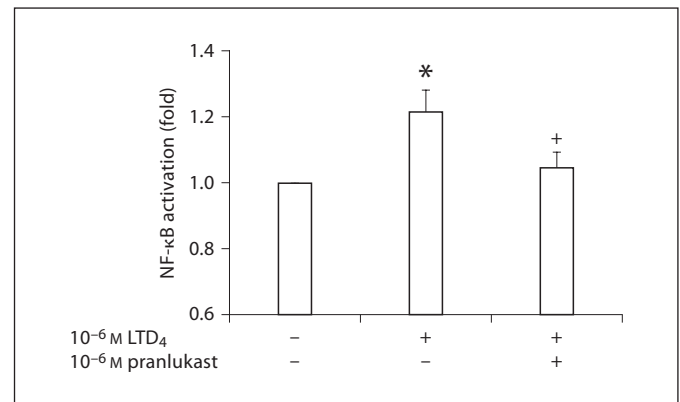


Fig. 7. NF-κB activation induced by LTD₄ stimulation in peripheral blood CD14+ monocytes/macrophages, as measured by ELISA. Cells are treated with LTD₄ for 10 min. Pranlukast was added 30 min before LTD₄ stimulation. The data (n = 8) are expressed as the ratio of stimulated cells to cells cultured in medium including 0.2% DMSO, and presented as means ± SD. * p < 0.05, compared to cells cultured in medium including 0.2% DMSO. + p < 0.05, compared to cells treated with LTD₄.

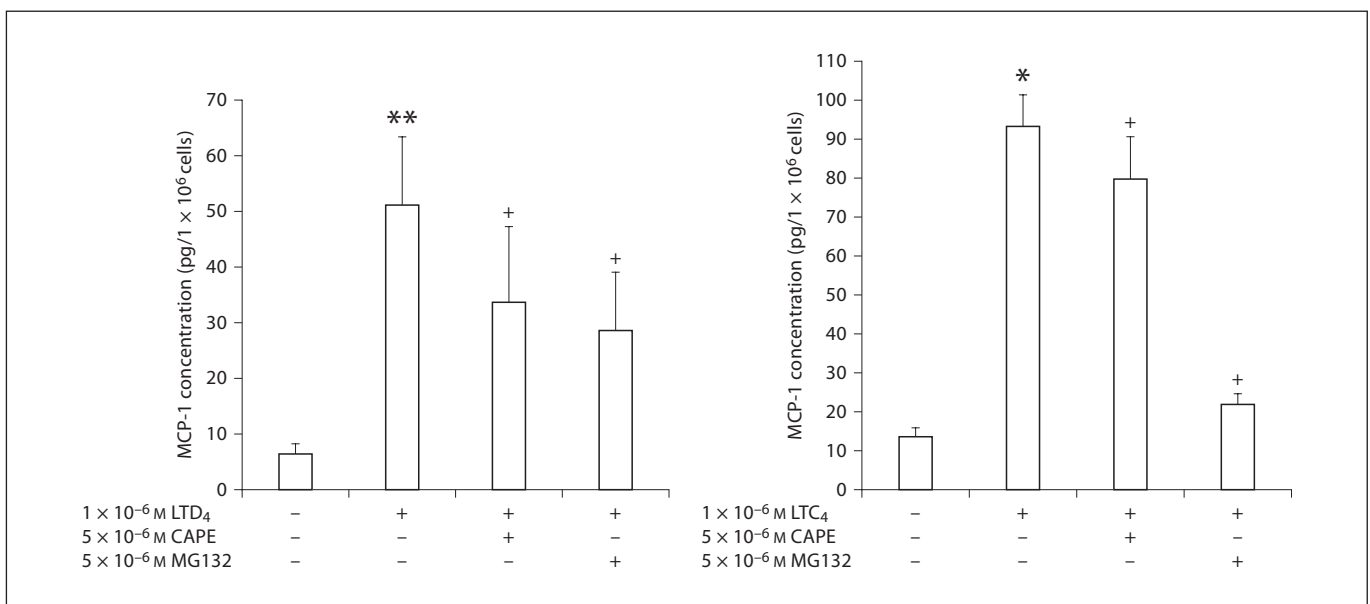


Fig. 8. Inhibitory effect of NF-κB inhibitors on MCP-1 release, as measured by ELISA, in THP-1 cells stimulated at 10⁻⁶ M LTD₄ or LTC₄ for 4 h. Cells were pretreated with 5 × 10⁻⁶ M CAPE and MG-132 for 30 min before LTD₄ or LTC₄ treatment. Data (n = 8)

are presented as means ± SD. ** p < 0.01 and * p < 0.05, compared to cells cultured in medium including 0.2% DMSO. + p < 0.05, compared to culture fluid of cells treated with LTD₄ or LTC₄.

ment of native T helper cells toward the Th2 phenotype [25]. Successful Th2 differentiation depends on the effectiveness of the T cell antigen receptor-mediated activation of the Ras/ERK pathway [26]. JNK activates a transcription factor activator protein-1, which encodes various immunomodulatory genes [27], suggesting an implication in the pathogenesis of asthma [28]. The p38 MAPK pathway is involved in IL-13-induced mucous cell metaplasia [29]. IgE production by B cells is mediated by p38 MAPK [30]. A previous study revealed that LTD₄ activated ERK1/2 pathway in THP-1 cells [16]. We showed that not only ERK1/2, but also JNK, phosphorylation was induced by LTD₄ and LTC₄ and that MCP-1 production was inhibited by inhibitors of both. These phosphorylations were observed 10 min after addition of LTD₄ and LTC₄, but not after 30 min. Our results coincided with previous studies showing that phosphorylations of MAPK proteins immediately occurred and were finished at once [31, 32]. The reason why the p38 MAPK inhibitor SB203580 significantly enhanced MCP-1 release induced by LTD₄ and LTC₄ from THP-1 cells is unknown. One hypothesis is that other intracellular signal pathways associated with MCP-1 production may be accelerated by blocking p38 MAPK. Our findings suggested that ERK1/2 and JNK pathways mediated MCP-1 production induced by LTD₄ and LTC₄ in human monocytes/macrophages. CysLTs may accelerate Th2 differentiation via ERK activation and activate activator protein-1 via JNK activation. In addition, pranlukast completely inhibited phosphorylations of ERK1/2 and JNK and MCP-1 production stimulated by LTD₄ and LTC₄ in human monocytes/macrophages, which suggested that phosphorylations of ERK1/2 and JNK as well as MCP-1 induction by CysLTs were mediated via a CysLT1 receptor.

NF- κ B binding to its consensus DNA binding site was increased in induced septum of asthma patients [33]. Nu-

clear extracts of bronchial biopsy specimens from asthma patients showed greater levels of NF- κ B-DNA binding [34]. NF- κ B was induced by inflammatory stimuli in human tracheobronchial epithelial cells [34]. These reports suggested that NF- κ B activation plays an important role in the pathogenesis of asthma. In the present study, NF- κ B activation was induced by LTD₄ and LTC₄ in THP-1 cells and peripheral blood CD14⁺ monocytes/macrophages, and MCP-1 production induced by LTD₄ and LTC₄ was inhibited by CAPE and MG-132 in THP-1 cells. The data suggested that the NF- κ B pathway mediated MCP-1 production by CysLTs in human monocytes/macrophages. Previous studies reported that LTD₄ induced NF- κ B activation in human epithelial cells [35] and in a murine model of asthma [36]. We demonstrated that NF- κ B activation was induced by LTD₄ and LTC₄ in human monocytes/macrophages. Pranlukast significantly inhibited NF- κ B activation induced by LTD₄ and LTC₄, suggesting that the activation was mainly mediated via CysLT1 receptor. Regarding inhibitory effects of CysLT receptor antagonists on NF- κ B activation, we previously demonstrated that CysLT receptor antagonists inhibited NF- κ B activation induced by tumor necrosis factor- α in human monocytes/macrophages [37, 38].

We have reported that CysLTs induced expression of MCP-1 and its receptor, CCR2B, and that CysLTs enhanced matrix metalloproteinase-9 which is responsible for the airway remodeling in human monocytes/macrophages [9, 39]. Our previous studies suggest that CysLTs are closely connected with the function of monocytes/macrophages in allergic inflammation. Our present study revealed one component of intracellular signal transduction mediated by CysLTs in human monocytes/macrophages. In conclusion, ERK1/2 and JNK in MAPK and NF- κ B pathways mediate MCP-1 production induced by CysLTs in human monocytes/macrophages.

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