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Cysteinyl leukotriene receptor antagonist epigenetically modulates cytokine expression and maturation of human myeloid dendritic cells

Chang-Hung Kuo [a, b, c, 1], San-Nan Yang [c, d, 1], Hsuan-Fu Kuo [e, f], Min-Sheng Lee [g, b], Ming-Yii Huang [h, i], Shau-Ku Huang [j, k, l], Yi-Ching Lin [m, n, o], Chong-Chao Hsieh [p, 2], Chih-Hsing Hung [g, j, q, r, s, *, 2]

[a] Ta-Kuo Clinic, Kaohsiung, Taiwan
[b] Department of Pediatrics, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung, Taiwan
[c] School of Medicine, College of Medicine, I-Shou University, Kaohsiung, Taiwan
[d] Department of Pediatrics, E-DA Hospital, I-Shou University, Kaohsiung, Taiwan
[e] Department of Internal Medicine, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung, Taiwan
[f] Division of Cardiology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[g] Department of Pediatrics, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[h] Department of Radiation Oncology, School of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[i] Department of Radiation Oncology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[j] Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[k] Johns Hopkins University School of Medicine, Baltimore, MD, USA
[l] National Health Research Institutes, Miaoli County, Taiwan
[m] Department of Laboratory Medicine, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[n] Department of Laboratory Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[o] Graduate Institute of Clinical Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[p] Division of Cardiovascular Surgery, Department of Surgery, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[q] Department of Pediatrics, School of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[r] Department of Pediatrics, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung, Taiwan
[s] Research Center of Environmental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

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ABSTRACT

Background: Cysteinyl leukotriene receptor antagonists are important controllers in treating asthma. Human myeloid DCs (mDCs) play critical roles in the pathogenesis of asthma. However, the effects of cysteinyl leukotriene receptor antagonist on human mDCs are unknown.
Methods: To investigate the effects of cysteinyl leukotriene receptor antagonist on the function of human mDCs, circulating mDCs were isolated from six health subjects. Human mDCs were pretreated with montelukast and were stimulated with toll-like receptor (TLR) ligands lipopolysaccharide (LPS) or polyinosinic-polycytidylic acid (poly I:C). Tumor necrosis factor (TNF)-α and interleukin (IL)-10 were measured by ELISA. Intracellular signaling was investigated by pathway inhibitors, western blot and chromatin immunoprecipitation. Costimulatory molecules expression was investigated by flow cytometry. T cell polarization function of mDCs was investigated by measuring interferon (IFN)-γ, IL-13, IL-10 and IL-17A production by T cells using mDC/T cell coculture assay.
Results: Montelukast suppressed TLR-mediated TNF-α expression via the NFκB-p65 and mitogen-activated protein kinase (MAPK)-JNK pathway, and enhanced TLR-mediated IL-10 expression via the MAPK-p38 pathway and epigenetic regulation by histone H3 acetylation. Montelukast suppressed LPS-induced CD80, CD86, CD40 and HLA-DR expression. Montelukast-treated mDCs suppressed IFN-γ and IL-13 production by T cells.
Conclusion: Cysteinyl leukotriene receptor antagonist alters the function of human mDCs by epigenetically modulating cytokine expression, suppressing costimulatory molecules expression and inhibiting

* Corresponding author. Department of Pediatrics, Kaohsiung Medical University Hospital, No. 100, Tz-You 1st Road, Kaohsiung, Taiwan.
E-mail address: pedhung@gmail.com (C.-H. Hung).
[1] Chang-Hung Kuo and San-Nan Yang contributed equally to the manuscript.
[2] Chong-Chao Hsieh and Chih-Hsing Hung contributed equally to the manuscript.

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the ability to initiate Th1/Th2 responses. The effects of cysteinyl leukotriene receptor antagonist on human mDCs can be an important mechanism in treating asthma.

1. Background

Asthma is a chronic airway inflammatory disorder with accumulation of inflammatory cells as well as inflammatory mediators, and the disease process is regulated by the modulation of interactions between antigen-presenting cells and T cells [1]. Tumor necrosis factor (TNF)-α is a pleiotropic pro-inflammatory cytokine, and increased TNF-α mRNA and protein levels has been reported in the airways of asthmatic patients [2]. Interleukin (IL)-10 has broad anti-inflammatory effects and is a feedback regulator of both T helper (Th) 1-type and Th2-type responses [3]. IL-10 inhibits cytokine production by inflammatory cells and also reduce airway inflammatory as well as hyperreactivity [4]. In severe refractory asthmatic patients, systemic production of IL-10 is significantly decreased [5].

Dendritic cells (DCs) are professional antigen presenting cells and are highly heterogeneous in terms of origin, morphology, phenotype, and function. DCs play major roles in initiation and regulation of adaptive immune responses to antigens [6]. Myeloid DCs (mDCs) are involved in the pathogenesis of asthma. In murine asthma model, mDCs accumulate in the allergen-challenged airways during the acute phase, and the depletion of mDCs attenuates the airway inflammation and hyperresponsiveness [7]. In human asthma, mDCs accumulate in bronchoalveolar lavage fluid after allergen challenge [8], and the influx of mDCs into the airways can be augmented by lipopolysaccharide (LPS) [9]. The induction and maintenance of inflammatory responses to allergen in persistent airway disease needs the involvement of mDCs [10]. These studies suggest the key role of mDCs in allergic airway inflammation.

Leukotriene is a key mediator of inflammatory processes and is involved in asthma and airway hyperresponsiveness [11]. Drugs inhibiting leukotriene signaling, such as cysteinyl leukotriene receptor antagonists (LTRAs), are effective in treating adult and childhood asthma [12,13]. LTRAs are now suggested as important controllers in treating asthma in Global Initiative for Asthma (GINA) guideline [14]. Recently the new applications of LTRAs are being under investigation [15]. However, there is a significant degree of interpatient variability in response to LTRAs treatment, and multiple pathways of anti-inflammatory actions independent of cysteinyl leukotriene receptor by LTRAs are therefore suggested [16].

In the present study, we investigated the *in vitro* effect of montelukast, a LTRA, on cytokine expression in mDCs and the intracellular mechanism including epigenetic regulation, which plays a significant role for the pathogenesis of asthma through the interaction of environments and genetic background [17]. The effects of montelukast on co-stimulatory molecule expression and T cell stimulatory function of mDCs were also studied.

2. Methods

2.1. Isolation and culture of mDCs

The study of human subjects was approved by the Institutional Review Board of Kaohsiung Medical University, Kaohsiung, Taiwan. Peripheral blood samples were obtained from healthy individuals ($n = 6$) after informed consents were obtained. Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation over Ficoll-Histopaque (Pharmacia Biotech, Uppsala, Sweden), and blood mDCs were magnetically sorted from PBMCs with BDCA-1 cell isolation kits (Miltenyi Biotec, Bergisch Gladbach, Germany). The purity was >90%. The isolated human mDCs were cultured in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin at 37 °C with 5% CO2 in a humidified incubator. Purified mDCs (10^5/well) were pretreated with varying doses of montelukast or ketotifen (10^{-8} M–10^{-5} M) for 2 h and were stimulated with toll-like receptor (TLR) 3 agonist, polyinosinic-polycytidylic acid (poly I:C; 10 μg/ml) or TLR 4 agonist, lipopolysaccharide (LPS; 0.2 μg/ml), or mite epitope (Der p2; 2.5 μg/ml; INDOOR Biotechnologies, Charlottesville, VA) in the presence of montelukast or ketotifen. Supernatants were collected at indicated time points.

To investigate the cell signaling, the cells were pretreated with IκB kinase (IKK) inhibitor BAY 117085 (Calbiochem, Cambridge, MA), mitogen-activated protein kinase (MAPK)-p38 inhibitor (SB203580), MAPK-JNK inhibitor (SP600125) or MAPK-ERK inhibitor (PD98059) for 1 h and then stimulated with LPS for 24 h. All MAPK inhibitors were purchased from Cayman Chemical Company (Cayman Chemical, Ann Arbor, MI, USA). Supernatants were collected at indicated time points.

2.2. Western blot

After treatment for 2 h with or without montelukast (10^{-5} M), the cells were stimulated with LPS 0.2 μg/ml for 1 h, and then lysed with lysis buffer. Equal amounts of cell lysates were analyzed by Western blot with anti-p65, anti-MAPK (p38, ERK and JNK), anti-phospho-p65 (pp65) and anti-phospho-MAPK (pp38, pERK and pJNK) antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Immunoreactive bands were visualized using horseradish peroxidase-conjugated secondary antibody and the enhanced chemiluminescence system (Amersham Pharmacia Biotech, Sunnyvale, CA, USA).

2.3. T cell stimulation assay

Autologous $CD4^+$ T cells were purified from PBMCs with human CD4 magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany). Human mDCs were pretreated with montelukast for 2 h and then stimulated by LPS for 48 h. The culture medium was washed out for 3 times, and the mDCs were cocultured with $CD4^+$ T cells (10^5 mDCs/10^6 T cells) in the presence of anti-CD3/CD28 antibodies for 3 days. Supernatants were collected for interferon (IFN)-γ, IL-13, IL-10 and IL-17A measurement.

2.4. Enzyme-linked immunosorbent assay (ELISA)

The level of cytokines (TNF-α, IL-10, IFN-γ, IL-13 and IL-17A) in the culture supernatants was determined by ELISA (R& D System, Minneapolis, MN, USA).

2.5. Flow cytometric analysis

Isolated mDCs were pretreated with montelukast (10^{-5} M) for