International Immunopharmacology 20 (2014) 81–88

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

The chemokine-like factor 1 induces asthmatic pathological change by activating nuclear factor-κB signaling pathway

Gang Li [a,b,*], Guang-yan Li [b], Zhen-zhen Wang [b], Hai-jie Ji [b], Dong-mei Wang [b], Jin-feng Hu [b], Yu-he Yuan [b], Gang Liu [b], Nai-hong Chen [b,**]

[a] Department of Pharmacology, College of Pharmacy, The Inner Mongolian Medical University, Jinshan Development Zone, Hohhot, Inner Mongolian 010110, China
[b] State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences, Peking Union Medical College, 1 Xiannongtan Street, Xicheng District, Beijing 100050, China

ARTICLE INFO

Article history:
Received 21 March 2013
Received in revised form 13 February 2014
Accepted 13 February 2014
Available online 25 February 2014

Keywords:
Chemokine-like factor 1
Asthma
NF-κB

ABSTRACT

CKLF1, which exhibits chemotactic activities on a wide spectrum of leukocytes, is up-regulated during the progress of asthma. It plays a vital role in the pathogenesis of pulmonary disease. Here, we report that CKLF1 has the capability to activate the NF-κB signaling pathway leading to the pathological change in the lung. The HEK293–CCR4 cell line, which expressed CCR4 stably, was established and screened. Western blot analysis was performed to determine the expression of NF-κB in HEK293–CCR4 and A549 cells following the C27 (10 μg/ml) added in each well at different times. These results showed that C27 (10 μg/ml) time-dependently induced the accumulation of NF-κB in the nucleus of HEK293–CCR4 and A549 cells. In addition, CKLF1 plasmid (100 μg) injection and electroporation led to the asthmatic change in the lung in mice as shown by HE and PAS staining. Furthermore, it was confirmed that CKLF1 significantly up-regulated the p-IκB expression, decreased the IκB expression, and suppressed the NF-κB expression in the cytoplasm of pulmonary tissue in vivo study. Intriguingly, an enhanced nuclear accumulation of NF-κB was observed in the lung of pCDI-CKLF1 electroporated mice, compared to that in the sham group. Therefore, the NF-κB signaling pathway was involved in the asthmatic change induced by CKLF1, among which CCR4 might play a crucial role.

1. Introduction

Asthma is characterized clinically with repeated episodes of wheezes and dyspnea, associated with, at least partially, reversible airway narrowing, physiologically increased airway responsiveness, pathological inflammation of the airway with the infiltration of eosinophils, T cells and mast cells, immunological response in around two thirds of patients, and increased production of IgE antibodies to environmental allergens [1]. The pathophysiology of asthma is complex and involves airway inflammation, intermittent airflow obstruction, and bronchial hyperresponsiveness. The development of chronic airway inflammation in asthma depends upon the continuous recruitment of inflammatory cells from the bloodstream towards the bronchial mucosa followed by their subsequent activation. The recruitment of immune cells into healthy tissues contributes importantly to the pathogenesis of asthma. The chemokine family of proteins is central to the regulation of selective leukocyte recruitment [2]. The chemokine is involved in a variety of inflammatory responses via interaction with chemokine receptors located on the cell surface of leukocytes followed by chemotaxis and infiltration into the adjacent tissue [3].

Chemokine-like factor 1 (CKLF1) is a novel human cytokine isolated from the PHA-stimulated U937 cells. The functional receptor of CKLF1 is the human chemokine receptor CCR4 [4,5]. The open reading frame of CKLF1 cDNA encodes a highly basic and hydrophobic polypeptide of a total of 99 residues with a calculated molecular mass of 10.9 Kd. CKLF1 is widely expressed in human tissues, it is highly distributed in the lung, peripheral blood leukocytes, etc. CCR4 are chemokine

Abbreviations: CKLF1, chemokine-like factor 1; C27, the 27 amino acid residual peptide of CKLF1; C19, the 19 amino acid residual peptide of CKLF1; FITC, fluorescein isothiocyanate; SARS, severe acute respiratory syndrome; NF-κB, nuclear factor kappa B; TNF-α, tumor necrosis factor-alpha; p-IκB, phosphorylation-inhibitory protein of NF-κB; HEK293, human embryonic kidney 293 cells; A549, human lung adenocarcinoma A549 cells; pCDI-CKLF1, plasmid pCDI CKLF1; SDF-1, stromal cell-derived factor-1; CCR4, CC chemokine receptor 4; TARC/CCL17, thymus and activation-regulated chemokine; MDC/CCL22, the macrophage-derived chemokine HEK293–CCR4, HEK293 cells express CCR4 stably; CXCR4, CXC chemokine receptor 4; PBMCs, peripheral blood mononuclear cells; RPMI-1640, Roswell Park Memorial Institute 1640; FBS, fetal bovine serum; PBS, phosphate buffer saline; Th2 cells, T helper type 2; IL-4, interleukin 4; IL-5, interleukin 5; IL-13, interleukin 13; WBC, white blood cell; DEX, dexamethasone; BLAF, bronchoalveolar lavage fluid; MDC, macrophage-derived chemokine; TARC, thymus and activation-regulated chemokine.

* Correspondence to: G. Li, Department of Pharmacology, College of Pharmacy, The Inner Mongolian Medical University, Jinshan Development Zone, Hohhot, Inner Mongolian 010110, China. Tel.: +86 472 6653134.
** Correspondence to: N. Chen, State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, 1 Xiannongtan Street, Xuanwu District, Beijing 100050, China. Tel./fax: +86 10 63165177.
E-mail addresses: cnnmligang@hotmail.com (G. Li), chennh@imm.ac.cn (N. Chen).

http://dx.doi.org/10.1016/j.intimp.2014.02.014
1567-5769/

receptors important to allergic rhinitis, a condition whose pathogenesis is similar to that of asthma, and it plays an essential role in the recruitment of Th2 cells into the airway upon the allergen stimulation. The CKLF1 binding motif to CCR4 has been characterized as a 27 amino acid residual peptide (ALIYRKLLFNPSGPYQKKPVHEKKEVL, C27), and the chemically synthesized C27 can also interact with CCR4 [6]. CKLF1 exhibits a potent chemotactic activity on a wide spectrum of leukocytes, both in vitro and in vivo [7], stimulates the proliferation of murine skeletal muscle cells after in vivo administration of naked CKLF1, and promotes the proliferation of the rat aortic smooth muscle cells [7]. CKLF1 plasmid transformation caused a pathological change in the seminiferous tubules in rats leading to infertility [8,9]. Besides, CKLF1 stimulates the proliferation of human bone marrow hematopoietic progenitor cells and colony formation [10]. It has been reported that CKLF1 expression is increased in inflammation and autoimmune diseases like rheumatoid arthritis and asthma [4,11,12]. Especially in asthma, the gene expression of CKLF1 in the lungs of asthmatic animal group is significantly increased compared to the control group. The level of CKLF1 mRNA in the peripheral blood mononuclear cells (PBMCs) from asthma patients is much higher than that from health donors. Moreover, a single intramuscular injection of CKLF1 plasmid DNA into BALB/c mice causes dramatic pathological changes in the lungs of treated mice. These changes include peribronchial leukocyte infiltration, epithelial shedding, collagen deposition, proliferation of bronchial smooth muscle cells and fibrosis of the lung, which are similar to the observation in chronic persistent asthma and SARS [13].

Since the pathophysiological mechanism underlying CKLF1-induced asthma remains unknown, based on the abovementioned analysis, we hypothesized that the NF-κB signaling pathway might take part in the progress. The purpose of this study is to elucidate the mechanism of the asthmatic pathological change trigged by CKLF1.

2. Materials and methods

2.1. Cell culture

HEK293 cells and A549 cells, obtained from the American Tissue Type Collection (ATCC), were grown respectively in 75 cm^2 tissue culture flasks in RPMI-1640 medium supplemented with 10% FBS and penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37 °C 95% air–5% CO_2 in a humidified incubator.

2.2. Mice

BALB/c mice were purchased from the National Institutes for Food and Drug Control (Beijing, China). All mice were housed in a specific pathogen-free facility at our laboratory. Male mice were used for all experiments. All mice were maintained on a 12:12 light:dark cycle at a temperature of 22 ± 2 °C with a humidity of 50 ± 10%, fed with regular mice chow and allowed tap water ad libitum. All experiments were approved by the Animal Care and Use Committee of Chinese Academy of Medical Sciences and carried out in accordance with the European Commission Directive 86/609/EEC for animal experiments. We used the minimum number of animals and made every effort to reduce animal suffering.

2.3. Antibodies and reagents

The CKLF1 for rat poly-antibody IgG was obtained from Human Disease Genomics Center of Peking University (Beijing, China). The CCR4 rabbit polyclonal IgG, p-IκB-α rabbit polyclonal IgG, IκB-α mouse monoclonal IgG, and NFκB (P65) mouse monoclonal IgG were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Horseradish peroxidase-conjugated goat anti-rabbit IgG and FITC labeled goat anti-rabbit IgG were obtained from Zymed Laboratories (CA, USA). The CKLF1 peptide (Aliyrkllfnpsgpyqkkpvhekkevl, CKLF-27) with purity greater than 95% was provided by GL Biochem (Shanghai) Ltd. (Shanghai, China). RPMI-1640 medium and fetal bovine serum (FBS) were purchased from Gibco BRL (New York, USA). The nucleus extraction kit was purchased from Neo-Bioscience Technology Company Limited (Shenzhen, China).

2.4. Transfection

The human pcDI-CCR4 and pcDI vector were transfected into the HEK-293 cells by using Lipofectamine™ 2000 (Invitrogen Corp. California, USA) according to the manufacture's protocol, respectively. In order to maintain CCR4 expression, 48 h later, HEK-293 cells were treated with geneticin (G418) at the final concentration of 500 μg/ml for the following culture. Then the monoclonal pool of stable transfected cell was selected and propagated. The expression of CCR4 was confirmed by western blot analysis.

2.5. Immunocytochemistry

The HEK293–CCR4 cells and mock-HEK293 cells were collected, coated on coverslips, and fixed with 4% paraformaldehyde for 15 min before antibody staining. Then the slides were rinsed with phosphate-buffered solution (PBS) thrice and blocked the unspecific antibody binding at room temperature with 10% normal goat serum for 1 h. The cells were incubated with 1:100 dilution of anti-CCR4 (1:100 dilution) antibody in PBS containing 5% goat serum for 2 h at room temperature, then followed by incubating for 1 h with FITC-conjugated goat anti-rabbit IgG (1:100) at room temperature. Finally, the cells were exposed to Hoechst 33458 for 15 min after 3 times of wash, then the coverslips were mounted on microscope slides and the fluorescence image was obtained by a confocal microscope (Leica TCS SP2, Ex = 490 nm, Em = 530 nm).

2.6. CKLF1 plasmid injection with electric-pulse delivery

The mice were treated as described previously [13]. Briefly, the mice were anesthetized by chloral hydrate, 100 μg of pCDB-CKLF1 in 100 μl of 0.9% NaCl was injected into the tibial cranial muscles of anesthetized mice, and the sham group was injected with 100 μg pCDB plasmid. There were 12 mice in each experimental group. At defined times after DNA injection (25 s or 1 min, depending on the experiment), transcutaneous electric pulses were applied by two implanted stainless steel needles (0.08 mm in diameter, 3.50 mm in length; the depth of insertion was 2 cm; the gap between the two needles was 0.9 cm), followed by electroporation (40 ms, 100 V, Electro Square Porator TM, BTX, USA). Four weeks after CKLF1 plasmid electroporation, dexamethasone was administered intraperitoneally once daily at 10 mg/kg body weight and lasted for 14 days.

2.7. Collection and analysis of BALF cells

After blood collection, tracheas were exposed by cannulating upper tracheas and BALF was collected by lavaging twice with 1 and 0.8 ml of PBS (85–90% of the input volumes were recovered). Collected lavage fluid was centrifuged at 400 ×*g* for 5 min at 4 °C. After centrifugation, total cell was collected for differentiation by the ADVIA 120 hematology system (Bayer, USA).

2.8. Western blot analysis

Cells were scraped and lysed in a lysis buffer (pH = 8.0) containing 50 mM Tris-Cl, 150 mM NaCl,1% NP-40, 1 mM PMSF, 1 mM EDTA, 1 mM DTT, 1 μg/ml aprotinin and 1% Triton X-100 on ice. The cell lysates were solubilized in SDS sample buffer and separated by 9% SDS-PAGE, then transferred to PVDF membrane (Millipore). The membrane was blocked with 3% BSA and incubated with anti-CCR4 antibody, then followed by