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Original article

Peripheral blood mononuclear cell NF-κB p105 mRNA decreases during asthmatic attacks

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Abstract

Background: NF-κB is a transcription factor involved in expression of many inflammatory cytokines, chemical transmitters, and adhesion molecules. It has been reported to play a major role in the pathogenesis of asthma. NF-κB p50, which is the actual subunit that results from the cleavage of p105, is required for the induction of eosinophilia via IL-5 and chemokines.
Methods: The subjects were 10 patients with a mean age of 59.3 years (14–82 years). NF-κB p105 mRNA in peripheral blood mononuclear cells during the presence or absence of asthmatic attacks was investigated. Total RNA was extracted from peripheral blood mononuclear cells. After cDNA was synthesized using random primers, NF-κB p105 mRNA level was measured by real-time polymerase chain reaction.
Results: The NF-κB p105 mRNA level in peripheral blood mononuclear cells was lower during asthmatic attacks than in the absence of attacks, showing a significant difference (Wilcoxon's signed rank test: $p < 0.01$).
Conclusions: A drop in NF-κB p105 during an asthma attack could result in increased NF-κB activity. There is a possibility that a change in the NF-κB p105 mRNA level might indicate some pathogenetic state in bronchial asthma attacks.

Keywords: Bronchial asthma; NF-κB; Peripheral blood mononuclear cell (PBMC)

1. Background

The transcription factor, nuclear factor-kappaB (NF-κB), is a homo- or heterodimer consisting of subunits from the Rel family of proteins, which consists of c-Rel, NF-κB1 (p50), NF-κB2 (p52), Rel A (p65), and Rel B [1–5]. Within resting cells, NF-κB is retained in the cytoplasm, complexed to an inhibitor protein from the inhibitor of κB (IκB) family.

NF-κB is cleaved and released by various activation signals such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) [6,7], interleukin-2 (IL-2), leukotriene B4 (LTB4), allergens [8–10], mitogens, lipopolysaccharide, viral infection [11–13], oxidative stress [14], and exposure to reactive oxygen [15]. Stimulation activates the IκB kinase (IKK) complex, which then phosphorylates IκB. This allows NF-κB to translocate into the nucleus where it can bind to κB sequences in the promoters of NF-κB-dependent genes to upregulate transcription. NF-κB regulates the genes encoding intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E selectin, granulocyte–macrophage colony-stimulating factor (GM-CSF), TNF-α, interleukin-6 (IL-6), and chemokines belonging to the C-C and C-X-C

Abbreviations: cDNA, complementary DNA; CRP, C-reactive protein; CTR, carboxy-terminal region; EDTA, ethylenediaminetetraacetic acid; GINA, global initiative for asthma; GM-CSF, granulocyte–macrophage colony-stimulating factor; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; IκB, inhibitor of κB; HDAC, histonedeacetylase; ICAM-1, intercellular adhesion molecule-1; IgE, immunoglobulin E; IKK, IκB kinase; IL, interleukin; LTB4, leukotriene B4; mRNA, messenger RNA; NF-κB, nuclear factor-κB; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; RAST, radioallergosorbent test; RHD, rel-homology domain; RIST, radioimmunosorbent test; TAD, transcriptional activation domain; TIA, turbidimetric immunoassay; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1.

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families [16,17]. In addition, it has been reported that NF-κB is involved in interleukin-4 (IL-4) signaling and CD40 signaling, which are important for the immunoglobulin E (IgE) production process [18–20], and that NF-κB plays a protective role against apoptosis [21–24], showing a variety of functions.

The involvement of NF-κB in bronchial asthma has attracted attention [25–28]. In patients with bronchial asthma, NF-κB activity is elevated in airway epithelial cells, submucosal cells, and macrophages from induced sputum [29]. In NF-κB/Rel family member knockout mice, antigen challenge causes neither pulmonary inflammation nor airway hypersensitivity [30], and mice deficient in the p50 subunit of NF-κB are incapable of mounting eosinophilic airway inflammation [31]. Various antiasthmatic agents have been reported to affect NF-κB. Steroids inhibit activation and transfer of NF-κB to the nucleus by increasing IκB production, and inhibit cytokine production by blocking NF-κB-induced protein induction [32,33]. Although fluticazone did not exhibit any action [34], budesonide [35], formoterol [35], theophylline [36–38], pranlukast [39] and erythromycin [40] have been reported to inhibit activation of NF-κB in local airway sites, airway epithelial cells, and monocytes. These reports suggest that NF-κB is closely related to the pathogenesis of asthma.

While many reports suggest the importance of the role of lymphocytes in bronchial asthma, there has been no report on NF-κB in lymphocytes during bronchial asthma attacks. Although the p105 precursor has been postulated to play a role in the regulation of Rel/NF-κB activity, its physiological relevance remains unclear. p50 is the actual subunit that results from cleavage of the carboxyl terminal of p105. We measured and evaluated the level of NF-κB p105 messenger RNA (mRNA) in peripheral blood mononuclear cells (PBMC) in patients with bronchial asthma.

2. Methods

2.1. Blood sampling protocol

The study was prospective, and patients who suffered bronchial asthma attacks were enrolled. NF-κB mRNA level in PBMC was quantified during the presence and absence of asthmatic attacks in patients with bronchial asthma being treated as outpatients, after obtaining informed consent. The presence of asthmatic attacks when the patients attended was determined physically by hearing piping rales because of paroxysmal difficulty in breathing and wheezing. Classification of asthma severity and the severity of asthma attacks was defined by the Global Initiative for Asthma (GINA) 2006 guidelines. Patients in whom IgE radioimmunosorbent test (RIST) result was 170 IU/ml or more or who had a positive IgE radioallergosorbent test (RAST) for one or more of Japanese cedar, cat epithelium, dog epithelium, *Aspergillus*, *Candida*, *Alternaria*, cockroach, chironomidae, mite or house dust were defined as atopic type. Patients with both IgE RIST lower than 170 IU/ml and negative IgE RAST were defined as non-atopic type.

The patients had not received additional treatment other than inhalation of short-acting β-stimulants for 2 or more weeks before the attacks. Blood sampling during bronchial asthma attacks was done before treatment of the attacks. After improvement of asthma attacks, blood sampling was performed again. Absence of attacks was defined as absence for 2 or more weeks without additional treatment. To minimize the influence of the inhibitory effects of various antiasthmatic agents on activation of NF-κB, asthma maintenance medication was not changed before or after the attacks.

2.2. Measurement of NF-κB mRNA

First, 2 ml of peripheral blood was collected from the patients during the presence and absence of asthmatic attacks into ethylenediaminetetraacetic acid (EDTA)-2Na-containing blood sampling tubes. Blood samples were centrifuged, and the buffy coat was recovered. Mixed erythrocytes were lysed with lysing buffer, the samples were washed, and PBMC were obtained.

NF-κB mRNA was detected using a real-time polymerase chain reaction (PCR) method. Total RNA was extracted from PBMC by the acid guanidinium thiocyanate–phenol–chloroform extraction method using RNAzolB (Sawady, Tokyo, Japan), and was collected from the precipitate in ethanol. Complementary DNA (cDNA) was synthesized with random primers. The PCR reaction mixture was prepared using TaqMan Universal Master Mix (PE Applied Biosystems, CA, USA). The primer set to amplify NF-κB mRNA was designed according to Genbank NM003998, using primers exons 18–19: 5′-CAG GCT GCC TGC TCC TGG AG-3′ and exons 20–21: 5′-CCT GCA TTT TCC CAA GAG TCA TCC-3′. The probe (exon 19: 5′-AGA GGG TCC ACC AGG CTG GCA GCT C-3′) was designed to target an internal region between the forward and reverse primers. The primer set for amplification of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA, as an internal control, was designed according to Genbank M33197, using primers exon 7: 5′-TGC ACC ACC AAC TGC TTA GCA CCC-3′ and exon 8: 5′-CTT GAT GTC ATC ATA TTT GGC AGG-3′. The probe of G3PDH was designed at exons 7–8: 5′-TGA CCA CAG TCC ATG CCA TCA CTG C-3′. Each PCR reaction was performed for 50 cycles (95 °C for 30 s, 60 °C for 40 s, 72 °C for 30 s) using a real-time PCR system (ABI PRISM 7700 Sequence Detection System: PE Applied Biosystems). The PCR products of NF-κB mRNA were purified using a High Pure PCR Product Purification kit (Roche Molecular Biochemicals Diagnostic, IN, USA), and were directly sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems) with an ABI PRISM 3100 Genetic Analyzer (PE Applied Biosystems). The sequence was finally compared with each target mRNA sequence.

2.3. Measurement of CRP

C-reactive protein (CRP) was measured by turbidimetric immunoassay (TIA).