

Production of TNF- α by Peripheral Blood Mononuclear Cells through Activation of Nuclear Factor κ B by Specific Allergen Stimulation in Patients with Atopic Asthma

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ABSTRACT

Stimulation by specific allergens induces inflammatory cytokine production from peripheral blood mononuclear cells (PBMCs) in patients with atopic asthma, but the mechanism remains unknown. PBMCs were collected from six patients with atopic asthma with an immunoglobulin E–radioallergen sorbent test score to *Dermatophagoides farinae* of ≥ 4 and six nonatopic healthy subjects (score = 0) using a dish adhesion method after density gradient centrifugation. CD23 expression in PBMCs was analyzed by the fluorescence-activated cell sorting method. PBMCs were incubated with *D. farinae* or lipopolysaccharide, and production of tumor necrosis factor (TNF) α into the supernatant was measured by enzyme-linked immunosorbent assay. After incubation, immunostaining of the PBMCs with anti-nuclear factor κ B (NF- κ B) antibody (anti-p65 antibody against p65 as the subunit of NF- κ B)

was performed, and NF- κ B activation in extracted nuclear protein was examined by electrophoretic mobility shift assay. CD23 expression was significantly higher in PBMCs from patients with asthma than in the controls ($p < 0.01$). There was no significant difference in TNF- α production by lipopolysaccharide stimulation between the two groups, but *D. farinae*-specific TNF- α production was significantly higher in subjects with asthma than in the controls ($p < 0.05$). A significant translocation of NF- κ B to nuclei by *D. farinae* stimulation was observed in cells from subjects with asthma ($p < 0.01$). Our results indicated that TNF- α production was induced by *D. farinae* in PBMCs of patients with atopic asthma by the activation of NF- κ B via CD23. In patients with atopic asthma, CD23-mediated signals may cause proinflammatory cytokine production, which may lead to airway inflammation. (Allergy and Asthma Proc 24:19–26, 2003)

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Bronchial asthma is a chronic inflammatory disease of the airway characterized by airway obstruction and hypersensitivity, in which inflammatory cells such as macrophages, eosinophils, lymphocytes, and mast cells are involved.¹ In atopic asthma, various chemical mediators are produced and released from mast cells by an antigen-antibody reaction of immunoglobulin E (IgE) antibody binding to high affinity IgE receptors (Fc ϵ RI) on mast cells and allergens, resulting in a contraction of airway smooth muscles and an increase of microvascular leakage. IgE receptors

TABLE I

Characteristics of Patients with Atopic Asthma and Normal Subjects

No.	Sex	Age (yr)	IgE (IU/mL)	RAST Score (mite)	Asthma Severity	PC20 (mg/mL)
Patients with Atopic Asthma						
1	M	24	1050	6	Mild intermittent	4
2	M	29	590	4	Mild intermittent	8
3	F	30	1200	5	Mild intermittent	4
4	M	28	2400	6	Mild persistent	1
5	M	26	2300	6	Mild persistent	1
6	M	29	2640	6	Moderate	0.5
Mean $\pm$ SEM		27.7 $\pm$ 2.3	1696.7 $\pm$ 853.0			
Normal Subjects						
7	F	34	1.6	0	—	>16
8	M	26	7.9	0	—	>16
9	M	25	39.5	0	—	>16
10	F	22	30.4	0	—	>16
11	M	35	17.9	0	—	>16
12	F	32	2.5	0	—	>16
Mean $\pm$ SEM		29 $\pm$ 5.4	16.6 $\pm$ 15.6			

consist of Fc ϵ RI and low affinity Fc ϵ RII, and Fc ϵ RII is divided further into Fc ϵ RIIa and Fc ϵ RIIb. The latter, also known as CD23, is primarily expressed in circulating monocytes, alveolar macrophages, B lymphocytes, and eosinophils and plays an important role in IgE-dependent reactions in the effector phase.² Fc ϵ RIIb is expressed in antigen-presenting cells (APCs) such as monocytes and macrophages, suggesting that these cells are not only involved in the production of inflammatory cytokines indirectly by presenting the antigens to T lymphocytes in the asthmatic airway but also in the establishment and maintenance of allergic inflammation by directly producing inflammatory cytokines as a result of allergen-specific activation mediated by the IgE antibody. However, the role of monocytes and macrophages in bronchial asthma and is not fully understood.

Expression of the genes involved in inflammation and immunoresponses is regulated by transcription factors. Nuclear factor κ B (NF- κ B) regulates the expression of important inflammatory mediators such as adhesion molecules (intercellular adhesion molecule 1 and vascular cell adhesion molecule 1), enzymes (inducible nitric oxide synthase and cyclooxygenase 2), proinflammatory cytokines (interleukin [IL]-1 β , tumor necrosis factor [TNF] α , IL-6, and granulocyte-macrophage colony-stimulating factor), and chemokines (IL-8, regulation on activation normal T cells expressed and secreted, macrophage inflammatory protein 1 α),^{3,4} and is believed to be closely involved in the establishment of airway inflammation in asthma.¹ Previous studies have shown that expression of NF- κ B is enhanced in macrophages in induced sputum and in airway epithelium of patients with asthma compared with healthy subjects.⁵ However, others have shown NF- κ B activation is less in

bronchoalveolar lavage cells from patients with asthma in comparison with healthy subjects after challenge using specific allergens.⁶ Therefore, the exact role of NF- κ B in asthma is yet to be established.

The purpose of this study is to clarify whether NF- κ B is activated *in vivo* by specific allergens, leading to the production of inflammatory cytokines in patients with atopic asthma. For this purpose, we used peripheral blood mononuclear cells (PBMCs) obtained from patients with atopic asthma who were considered to be actively sensitized by anti-*Dermatophagoides farinae* IgE antibody, rather than cell lines or monocytes from healthy subjects passively sensitized *in vitro*, and examined whether NF- κ B is activated upon the induction of TNF- α by specific allergens using immunocytological techniques and electrophoretic mobility shift assay (EMSA).

MATERIALS AND METHODS

Subjects

The subjects were six patients with atopic asthma who had been sensitized by *D. farinae*, and they were the outpatients seen in the Second Department of Internal Medicine, Nagasaki University Hospital, Japan. We also recruited six nonatopic healthy volunteers. All subjects were nonsmokers. Table I summarizes the background of the subjects. Diagnosis of asthma and grading of its severity was based on Global Initiative for Asthma guidelines.⁷ None of the patients was on steroids either orally or by inhalation at the time of the study, but all were being treated with occasional inhalation of β_2 -agonists. This study was approved by the ethics committee of the Nagasaki Univer-

sity Hospital, and informed consent was obtained from all subjects.

Measurement of the Serum IgE-Radioimmunosorbent Test and IgE-Radioallergosorbent Test

Venous blood was obtained and serum IgE-RIST (Radioimmunosorbent test) and IgE-radioallergosorbent test (RAST) were measured using fluoroenzyme immunoassay (FEIA; Capsulated hydrolic carrier polymer (CAP) RIST FEIA and CAP RAST FEIA; Pharmacia, Uppsala, Sweden).

Measurement of Airway Responsiveness to Methacholine

Methacholine challenge test was performed using the 2-minute tidal breathing method.⁸ Short-acting inhaled β_2 -agonists were withheld for at least 24 hours before the measurement of airway responsiveness. Subjects were administered aerosols of methacholine from a handheld nebulizer (clinical air pump, model REB 30REPC-Medicare 24, Medicare Co., Yokohama, Japan) by tidal breathing for 2 minutes with airflow rate set at 5 l/minute. Isotonic saline was inhaled first as a control, followed by doubling concentrations of methacholine from 0.031 to 16 mg/mL. Forced expiratory volume in 1 second (FEV₁) was measured after each inhalation with a spirometer (Superspiro, DISCOM-21FX; Chest M.I. Co., Tokyo, Japan). The percentage of fall in FEV₁ was calculated from the postsaline FEV₁. The test was continued until FEV₁ had fallen by >20% or until the maximum concentration of methacholine was administered. Airway sensitivity was expressed as the concentration of methacholine necessary to provoke a fall in FEV₁ of 20% (PC20). At the end of the test, airway obstruction was reversed with inhalation of albuterol. PC20 < 8 mg/mL was regarded as positive for airway hypersensitivity.

Preparation of PBMCs

Heparinized venous blood obtained from healthy subjects and from subjects with asthma was diluted with RPMI1640 plus 10% heat-inactivated fetal bovine serum, 100 U/mL of penicillin, and 100 μ g/mL of streptomycin (hereafter referred to as cRPMI). Mononuclear cells were separated by Ficoll-Hypaque density gradient centrifugation. Cells were washed twice in cRPMI, plated in cell culture Petri dishes, and incubated overnight at 37°C in 5% CO₂. After nonadherent cells were removed by washing the dishes three times with warm RPMI1640 medium, adherent cells were incubated with cold phosphate-buffered saline on ice for 15 minutes. The adherent cells were subsequently recovered by vigorous pipetting, washed, and resuspended in cRPMI. The viability of adherent cells was assessed using the trypan blue exclusion test (>98% viable). Contamination of endotoxins in cRPMI was examined by the Toxicolor test (SRL Co., Tokyo, Japan) and found to be under 50 pg/mL.

Flow Cytometric Analysis

Surface CD23 and CD14 expression was analyzed using a flow cytometer (FACScan; Becton Dickinson, Tokyo, Japan) by using phycoerythrin-conjugated anti-CD23 (0.1 mg/mL; PharMingen, San Diego, CA) and phycoerythrin-conjugated anti-CD14 (0.1 mg/mL; PharMingen) by standard procedures. CD14 expression in adherent cells was >90%.

Cell Culture

Cells were plated in 96-well plates at a density of 2×10^5 cells/well and incubated for 6 hours in the medium, 6 hours with 1 μ g/mL of crude extract of *D. farinae* (LSL, Inc., Sigma Chemical Co., St. Louis, MO), or 2 hours with 1 ng/mL of endotoxin lipopolysaccharide (LPS) from *Escherichia coli* (Sigma Chemical Co., St. Louis, MO) at 37°C under 5% CO₂. Contamination of endotoxins in *D. farinae* was found to be under 50 pg/mL examined by Toxicolor test. We selected a concentration of stimulants and culture periods based on their optimal effect as noted in a series of preliminary experiments. After incubation, the cell suspension was centrifuged and the supernatant was frozen at -80°C until use. The pellet was resuspended in cRPMI and cytospin preparations were prepared for immunostaining.

Measurement of TNF- α in Supernatants of Cultured Cells

TNF- α concentrations in the supernatants of 6 hours after medium stimulation, 6 hours after *D. farinae* stimulation, and 2 hours after LPS stimulation were measured using an enzyme-linked immunosorbent assay kit (Quantikine; R & D Systems, Inc., Minneapolis, MN). The sensitivity of measurement was 4.4 pg/mL.

Immunostaining for NF- κ B

Cytospin preparations were fixed in cold acetone. Endogenous peroxidase activity was blocked by incubating slides in methanol containing 3% H₂O₂ for 15 minutes, followed by washing in phosphate-buffered saline. After blocking with 10% normal rabbit serum for 20 minutes, sections were incubated for 2 hours at room temperature with a goat polyclonal antihuman NF- κ B p65 antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) at a dilution of 1×100 of a 200 μ g/mL solution. After the application of the primary antibody, we applied a biotinylated rabbit anti-goat antibody (Vector Laboratories, Burlingame, CA), followed by the addition of peroxidase-conjugated avidin for 30 minutes at room temperature. Chromogen-fast diaminobenzidine (Sigma Chemical Co.) was used for 5 minutes, and the slides were counterstained in hematoxylin and mounted. The slides were numbered and examined by three investigators in a blinded fashion. Counted areas of slides were selected randomly at a low magnification, 500 PBMCs were examined for nuclear

staining, and positive cells scored at a high magnification ($\times 1000$) using an oil immersion lens. The mean values of the scores reported by the three investigators were calculated for each slide, and the results were expressed as percentages of cells with positive nuclear staining. Inter- and intraoperative variations were $<10\%$.

Nuclear Protein Extraction and Electrophoretic Mobility Shift Assay

Nuclear proteins were extracted using the method described by Abmayr *et al.*⁹ Cell lysis was induced with NP-40 (Sigma Chemical Co.) at 4°C for 3 minutes, and soluble nuclear extracts were obtained after osmotic lysis of the nuclear envelope. Nuclear proteins ($10\ \mu\text{g}$) were incubated at room temperature for 20 minutes with ^{32}P -labeled double-stranded oligonucleotides containing a tandem repeat of the consensus sequence for the NF- κB DNA binding site 5'-AGT TGA GGG GAC TTT CCC AGG C-3'. The binding reaction was analyzed by electrophoresis in a non-denaturing 6% polyacrylamide gel and visualized by autoradiography. To identify the specificity and components of NF- κB , PBMCs from patients with asthma were stimulated with LPS, and $1\ \mu\text{g}$ of nuclear protein taken from these cells was used in the following experiments. Specificity was determined by the addition of a 100-fold excess of unlabeled double-stranded NF- κB oligonucleotide. To confirm the identity of the components of retarded complexes, supershift experiments were conducted using anti-p50 and anti-p65 human antibodies (Santa Cruz Biotechnology, Inc.)

Statistical Analysis

Data are presented as mean $\pm$ SEM. Differences between subjects with asthma and subjects without asthma were compared by two-group unpaired *t*-test. Differences in the same patient between measurements performed before and after stimulation were compared by two-group paired *t*-test. A value of $p < 0.05$ was considered significant.

RESULTS

CD23 Expression on Monocytes

Expression of CD23 on monocytes was analyzed by fluorescence-activated cell sorting (FACS) and found to be significantly higher in patients with atopic asthma than in healthy subjects (Fig. 1).

Production of TNF- α by *D. farinae* Stimulation

In monocytes collected from healthy subjects, there was no significant difference in TNF- α production between medium stimulation and *D. farinae* stimulation. In monocytes collected from patients with atopic asthma, TNF- α production was significantly higher with *D. farinae* stimulation than with medium stimulation (Fig. 2 A). With non-specific LPS stimulation, significant increases in TNF- α

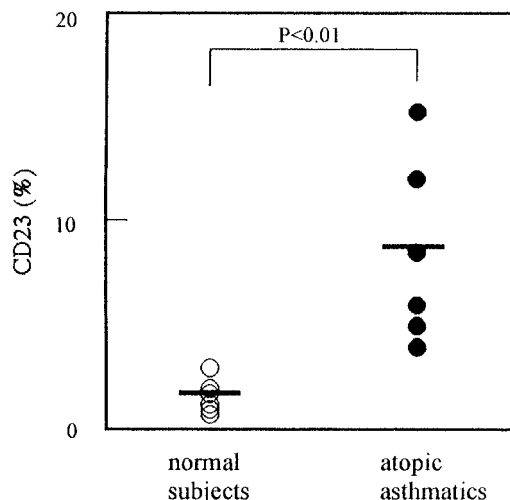


Figure 1. Proportion of CD23-expressing human monocytes. Expression of CD23 on monocytes was estimated by flow cytometry. The data represent percentages. Compared with normal subjects, the percentage of CD23 expressing monocytes was significantly higher in patients with atopic asthma. Horizontal bar represents the mean value of each group.

production were observed compared with medium stimulation in both healthy subjects and patients, and there was no significant difference in the level of TNF- α production between healthy subjects and patients with asthma (Fig. 2 B).

Translocation of NF- κB

Cells were subjected to immunostaining with an antibody against the p65 subunit of NF- κB after incubation. Figure 3 shows photographs of representative stained samples. In patients with asthma, PBMCs after medium stimulation showed very few nuclei stained with the antibody (Fig. 3 A), whereas after *D. farinae* stimulation, most cells showed positive staining (Fig. 3 B). Cells with nuclear staining were scored at $1000\times$ magnification and the percentage of positive cells was calculated (Fig. 4). In healthy subjects, no nuclear translocation was observed with *D. farinae* stimulation, but significant nuclear translocation was noted with LPS stimulation. In asthma patients, significant nuclear translocation was clear with both *D. farinae* and LPS stimulation.

NF- κB DNA Binding Activity

To examine the NF- κB DNA binding activity, nuclear protein ($10\ \mu\text{g}$) extracted from the cells after incubation by the mini-extract method was reacted with γ - ^{32}P -labeled NF- κB consensus oligonucleotide and subjected to electrophoresis (Fig. 5 A). In samples from healthy subjects, the NF- κB band was detected at about the same level with medium stimulation and *D. farinae* stimulation, whereas the band with LPS stimulation was more intense. In patients with atopic asthma, the band was thicker with *D. farinae* or

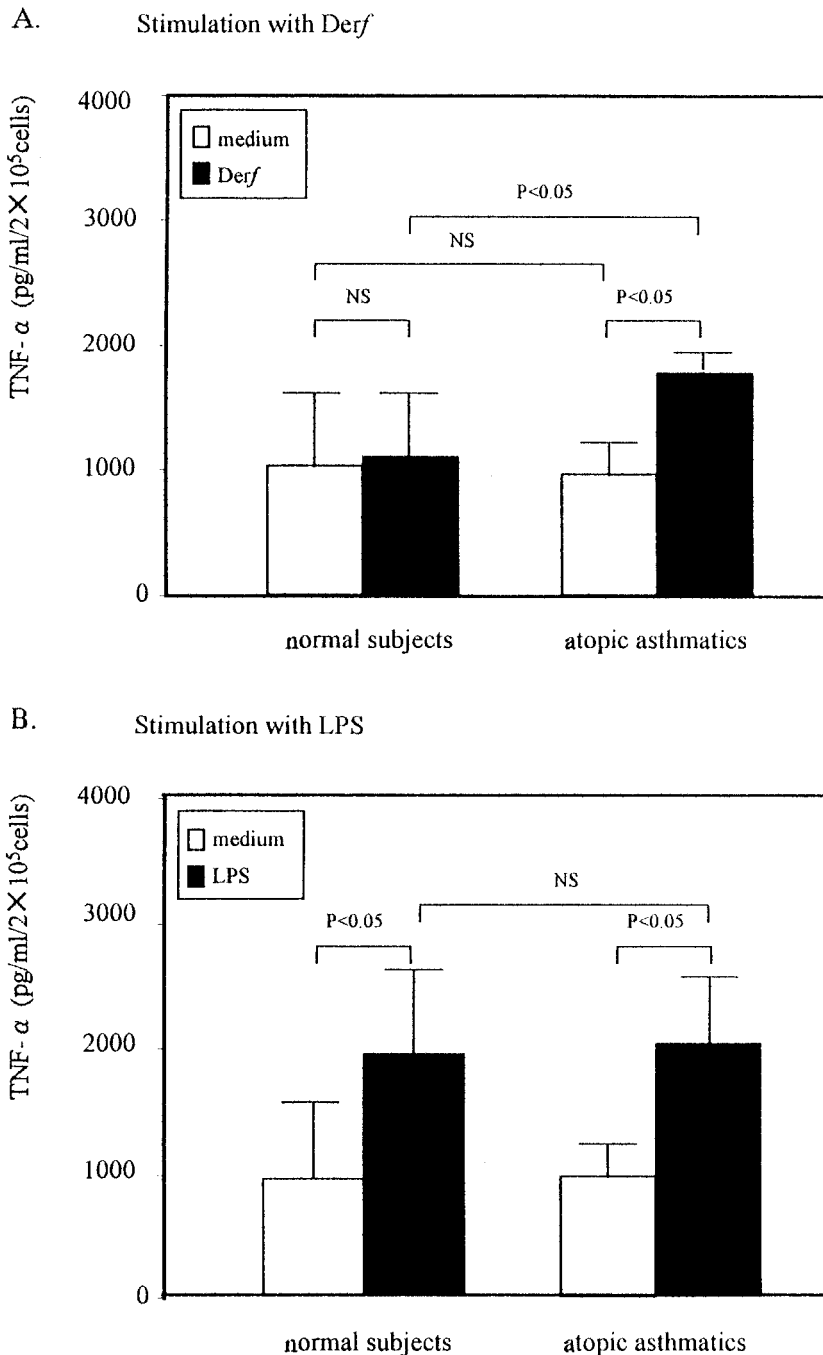


Figure 2. Production of TNF- α by human monocytes. The production of TNF- α from monocytes was estimated immunologically by the treatment with (A) *D. farinae* and (B) LPS. (A) In monocytes of normal subjects, no significant increase in TNF- α production was noted after medium and *D. farinae* stimulation. However, in monocytes of patients with atopic asthma, TNF- α production was significantly increased after *D. farinae* stimulation compared with medium stimulation. (B) TNF- α production was significantly increased after LPS stimulation in normal subjects and patients with asthma. There were no significant differences between the two groups in LPS-induced TNF- α production. Data are mean $\pm$ SEM.

LPS stimulation than with medium stimulation, in agreement with the results of TNF- α production and immunostaining. To confirm the specificity of the bands and to identify the activated subunit of NF- κ B, nuclear protein was extracted from LPS-stimulated monocytes from patients with atopic asthma, and 1 μ g of the protein was subjected to

the binding assay (Fig. 5 B). Addition of a 100-fold excess of unlabeled NF- κ B oligonucleotide, cold NF- κ B, eliminated the band, indicating its specificity. Supershift analyses with anti-p50 and anti-p65 antibodies both showed supershifts of the bands, indicating that the induced NF- κ B bands were composed of p50 and p65 subunits.

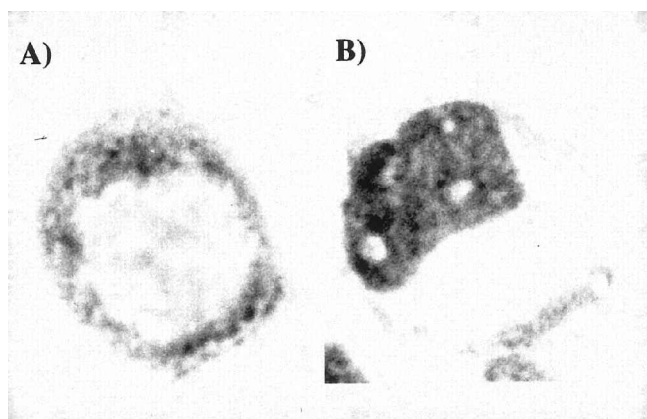


Figure 3. Immunostaining for NF- κ B in human monocytes in a patient with atopic asthma. Cultured cells were immunologically stained using an anti-p65 antibody. (A) The cytoplasm of monocytes obtained from a representative patient with atopic asthma was positively stained (magnification: $\times 1000$). (B) The nucleus of monocytes was positively stained 6 hours after *D. farinae* stimulation. These findings suggest that NF- κ B translocated from the cytoplasm to the nucleus of monocytes after *D. farinae* stimulation (magnification: $\times 1000$).

DISCUSSION

The major findings of this study were the following points. The expression of low affinity IgE receptor (CD23) is significantly enhanced in PBMCs of patients with atopic asthma compared with healthy subjects. Stimulation with *D. farinae* allergens induced production of signifi-

cantly larger amounts of TNF- α in PBMCs of patients with asthma. Specific stimulation with *D. farinae* allergens induced nuclear translocation of NF- κ B, as detected by immunohistological techniques and EMSA, in PBMCs of patients with asthma alone while nonspecific stimulation with LPS induced nuclear translocation of NF- κ B in PBMCs of both healthy subjects and patients with asthma. NF- κ B activated by *D. farinae* allergen stimulation consists of the p50 and p65 subunits.

Dust mites are an important source of the inhalant allergens and mite allergen exposures in infancy and childhood are a major risk factor for asthma.¹⁰ Mite allergen exposure also causes acute exacerbations of asthma *via* interaction between allergen and IgE antibodies binding to Fc ϵ RI on mast cells. Besides Fc ϵ RI, we examined the CD23-mediated inflammatory reactions in this study. The expression of CD23 on PBMCs obtained from mite allergen-sensitized asthma increased to a significantly higher level ($8.6 \pm 4.5\%$) than in healthy subjects ($1.6 \pm 0.7\%$). Williams *et al.*¹¹ also indicated that the expression level of CD23 in PBMC of asthmatic patients ($16.7 \pm 2.4\%$) was significantly higher than in normal subjects ($2.5 \pm 1.0\%$). The expression of CD23 is regulated by cytokines, and Th2 cytokines such as IL-4 and IL-13 enhance CD23 expression.¹² The Th2-dominant immunoresponse is one of the main pathogenesis events in asthma, and this is considered responsible for the increased expression of CD23 in patients with asthma. Because asthma consists of chronic airway inflammation, the study of the involvement of monocytes/

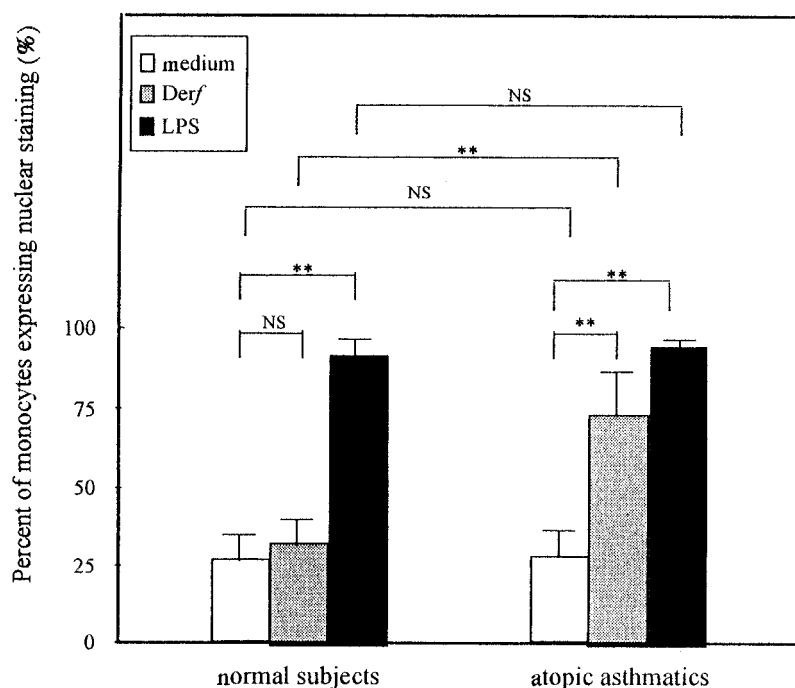


Figure 4. Proportion of monocytes expressing positive nuclear staining for NF- κ B. In normal subjects, nuclear translocation of NF- κ B was marked after LPS stimulation but not after *D. farinae* stimulation. In patients with atopic asthma, nuclear translocation of NF- κ B was marked after both LPS and *D. farinae* stimulation.

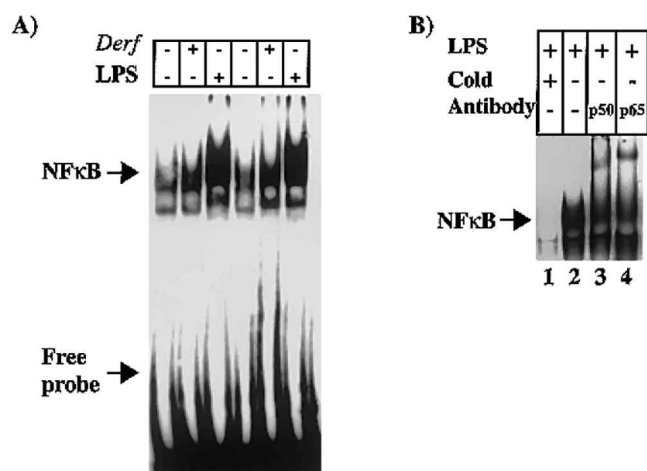


Figure 5. This figure shows EMSA. (A) Using the mini-extract method, nucleoproteins were extracted from cultured cells. γ - ^{32} P-labeled NF- κ B consensus oligonucleotide was reacted with 10 μ g of nucleoproteins under the following conditions. Lane 1, nonasthmatic PBMCs + medium; lane 2, nonasthmatic PBMCs + *D. farinae*; lane 3, nonasthmatic PBMCs + LPS; lane 4, atopic asthmatic PBMCs + medium; lane 5, atopic asthmatic PBMCs + *D. farinae*; lane 6, atopic asthmatic PBMCs + LPS. A band of NF- κ B was found after medium stimulation in cells from normal subjects. The band increased in intensity after LPS stimulation, although it did not change after *D. farinae* stimulation. A band of NF- κ B was also found after medium stimulation in cells from patients with atopic asthma. The band increased in intensity after LPS and *D. farinae* stimulation. (B) Nucleoproteins were extracted from monocytes after LPS stimulation in patients with atopic asthma. To investigate the specificity of NF- κ B, unlabeled NF- κ B oligonucleotide (cold NF- κ B) at an excessive 100-fold concentration was reacted with 1 μ g of nucleoproteins and analyzed with EMSA (lane 1). The specificity of NF- κ B was confirmed because the band disappeared under the condition. A supershift assay was performed using anti-p50 antibody (lane 3) and anti-p65 antibody (lane 4). The band of NF- κ B was supershifted by these antibodies. These results suggest that the induced band of NF- κ B consisted of p50 and p65 subunits.

macrophages in the pathogenesis of asthma is preferred to use of alveolar macrophages in the airway than PBMCs. Indeed, in the study by Williams *et al.*,¹¹ in subjects with asthma the proportion of CD23⁺ cells in alveolar macrophages was markedly higher ($69.2 \pm 6.3\%$) than that in PBMCs. It is considered that the limited expression of Fc ϵ RIIb on PBMCs reflects the degree of stimulation received during their passage through the lung or from mediators released from the lung into the circulation. Considering the risk of bronchoscopic examination and the possible effects of drugs administered before such tests, we used circulating monocytes in this study. It should be possible in the future to examine the molecular dynamics of CD23, TNF- α , and NF- κ B in the airway using induced sputum.

Ten *et al.*^{2,13} reported that passive sensitization with IgE followed by anti-IgE antibody stimulation of nonasthmatic monocytes or promonocytic cell line U937 with increased CD23 expression induced by preincubation with IL-4 caused activation of NF- κ B via CD23 cross-linking, resulting in the production of inflammatory cytokines. Because their experiments involved cell line or manipulated monocytes of healthy subjects, the results may not reflect real *in vivo* phenomenon in human asthma. In contrast, this study involved blood samples obtained from patients with asthma and showed that PBMCs from patients with atopic asthma, but not from nonatopic healthy subjects, show nuclear translocation of NF- κ B and produce a significant amount of TNF- α by specific allergen stimulation. Taken together, these results suggest the important role of monocytes/macrophages in the development of allergic airway inflammation in asthma. The Th2-dominant immunity in atopic individuals leads monocytes/macrophages to express CD23 on their surfaces. Specific allergen exposure induces CD23 cross-linking, which results in TNF- α production associated with nuclear translocation of NF- κ B. Interestingly, it has been shown that TNF- α induces NF- κ B expression in human monocytes, and NF- κ B regulates the transcription of TNF- α gene.⁴ This may be responsible for the perpetuation of chronic inflammation of the airway.

Establishment of allergic inflammation of the airway involves a number of molecules such as inflammatory cytokines, adhesion molecules, and lipid mediators. In normal human subjects, inhalation of recombinant TNF- α (60 ng) caused increased bronchial reactivity,¹⁴ and immunohistochemical analysis of bronchial biopsy specimens from patients with mildly allergic asthma revealed that the increase in TNF- α immunoreactivity correlated with airway hyper-responsiveness.¹⁵ TNF- α augments inflammatory cellular influx into the bronchial mucosa by up-regulating the expression of adhesion molecules on the microvasculature and subsequent recruitment and activation of inflammatory cells.¹⁶ We determined TNF- α in this study because this cytokine has been established to be involved in asthma as mentioned previously, it is produced by monocytes in IgE stimulation, and its transcriptional regulation involves NF- κ B.¹ Our results also showed that *D. farinae* allergen stimulation induced the production of significantly larger amounts of TNF- α in PBMCs collected from patients with asthma than from healthy subjects.

Glucocorticoids, which are currently the most effective drug for treatment of asthma, are potent inhibitors of NF- κ B¹⁷ but are associated with serious side effects and is ineffective in patients with steroid-resistant asthma. The PBMC incubation system, which was developed in this study, may allow the testing of the effects of existing anti-asthma drugs on NF- κ B and the development of selective inhibitors of NF- κ B, which should lead to substantial progress in the treatment of bronchial asthma and other allergic diseases.

In conclusion, this study suggests the distinct role of monocytes/macrophages in IgE-mediated allergic inflammation, wherein the Th2-dominant immunity up-regulates the expression of CD23, a low affinity IgE receptor, on monocytes/macrophages. The cross-linking of CD23 by IgE-allergen immunocomplexes leads to the activation of the transcription factor NF- κ B, resulting in the production of inflammatory cytokines including TNF- α . Monocytes/macrophages that are widely distributed in the bronchial mucosa are the first immune cells to encounter foreign antigens and initiate immunoreactions. Therefore, the production of TNF- α by monocytes in response to stimulation by specific allergens is considered a first step in the development of airway inflammation.

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