

Endogenous nitric oxide downregulates the Bcl-2 expression of eosinophils through mitogen-activated protein kinase in bronchial asthma

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Background: Eosinophil apoptosis might play a crucial role in the maintenance of persistent airway inflammation in asthma. Nitric oxide (NO) synthase activity is upregulated in eosinophils of asthmatic patients. Mitogen-activated protein kinase (MAPK) is implicated in regulating eosinophil apoptosis by modulating Bcl-2 expression. NO-induced cell apoptosis is associated with an inhibition of Bcl-2 expression.

Objective: We sought to study whether NO might induce eosinophil apoptosis through extracellular signal-regulated protein kinase (ERK) or p38 MAPK pathways and Bcl-2 expression.

Methods: Eosinophils were freshly isolated from peripheral blood of 16 asthmatic patients and 12 healthy subjects and then cultured with or without the NO synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME) at 1 and 10 mmol/L for 16 hours. The expression of Bcl-2 and induced NO synthase on eosinophils was analyzed by means of flow cytometry. Apoptosis was assessed by means of propidium iodide and DNA ladder. The activity of ERK and p38 MAPK was determined by means of Western blotting.

Results: The induced NO synthase immunoreactivities and the spontaneous release of nitrite from the eosinophils of asthmatic patients were higher compared with those of healthy subjects. Eosinophils of asthmatic patients were found to express more highly constitutive Bcl-2 than those of healthy subjects. After incubation for 16 hours, the expression of Bcl-2 on eosinophils from patients with asthma was significantly enhanced by L-NAME. The percentage of apoptosis was decreased by the addition of 1 mmol/L L-NAME in patients with asthma. The activity of p38 MAPK and ERK in eosinophils from patients with asthma was enhanced in the presence of L-NAME. An inhibition of MAPK reduced the Bcl-2 expression and increased eosinophil apoptosis in patients with asthma.

Conclusion: We concluded that inhibition of endogenous NO might increase the expression of Bcl-2 in eosinophils from patients with asthma through the signaling pathway of ERK

or p38 MAPK, which in turn decrease the apoptosis. (*J Allergy Clin Immunol* 2003;112:761-7.)

Key words: Eosinophils, apoptosis, nitric oxide, Bcl-2 protein, mitogen-activated protein kinase

Asthma is characterized by chronic airway inflammation, cellular infiltration, and subsequent cytokine production.^{1,2} Among the inflammatory cells, eosinophils play an important role in bronchial asthma.³ The number of eosinophils is increased in the airways of asthmatic patients and significantly correlates with the clinical severity of the disease.⁴

Nitric oxide (NO), a radical synthesized by nitric oxide synthase, has pleiotropic biologic activities and is implicated as a signaling, as well as a toxic, effector molecule.^{5,6} Patients with asthma and allergic rhinitis demonstrate increased NO levels in exhaled air.⁷ The inducible form of NO synthase (iNOS) is expressed in the bronchial mucosa of asthmatic patients⁸ but not of healthy subjects and can also be detected in blood eosinophils.⁹ We recently reported that an increased number of colonies of progenitor cells from asthmatic patients possess a higher proliferative capacity to develop into eosinophils,^{10,11} and the production of NO from progenitor cells or other circulating cells could suppress progenitor cell growth and colony formation.¹⁰ The pathophysiologic role of iNOS activity in peripheral blood eosinophils from patients with asthma is not yet known.

Apoptosis, a form of natural cell death, is characterized by condensation of nuclear chromatin, shrinkage of the cell, and separation of both nucleus and cytoplasm into multiple apoptotic bodies.^{12,13} Apoptosis is claimed to be fundamentally significant in the resolution of inflammation,¹⁴ and the induction of inflammatory cell apoptosis can terminate the airway inflammation of asthma. Reduction of inflammatory cell apoptosis, in particular that of eosinophils, might play a crucial role in the chronic persistence of airway inflammation in asthma. Apoptosis is regulated by various factors, such as Bcl-2 family proteins and p53. Bcl-2 family proteins regulate apoptosis, including antiapoptotic proteins, such as Bcl-2 and Bcl-x, and proapoptotic proteins, such as Bax and Bak.¹⁵ Both endogenous and exogenous NO has been shown to induce cell apoptosis associated with an inhibition of Bcl-2 expression¹⁶ in megakaryocytic cell lines and to sustain

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Abbreviations used

ERK: Extracellular signal-regulated kinase
 iNOS: Induced nitric oxide synthase
 L-NAME: N-nitro-L-arginine methyl ester
 MAPK: Mitogen-activated protein kinase
 MFI: Mean fluorescence intensity
 NO: Nitric oxide

increases in Bax expression in myocytes.¹⁷ Cells transfected with human Bcl-2 were resistant to NO-induced apoptosis.¹⁸ It is possible that endogenous NO produced by eosinophils might regulate eosinophil apoptosis mediated through an interaction with Bcl-2 expression.

The mitogen-activated protein kinases (MAPKs), which include extracellular signal-regulated protein kinase (ERK), and c-Jun N-terminal kinase/stress-activated protein kinase and p38 subfamilies are important regulatory proteins through which various extracellular signals are transduced into intracellular events.^{19,20} ERK and p38 MAPK play an important role in eosinophil differentiation, cytokine production, and degranulation.²¹ The Ras-MAPK kinase pathway is involved in the phosphorylation of Bad and the regulation of its function, which in turn modulates the expression of Bcl-2.²² The p38 MAPK activity is involved in granulocyte apoptosis and might be related to the Bcl-2-controlled pathway.²³

This study has demonstrated that Bcl-2 expression of peripheral blood eosinophils in asthmatic patients is regulated through the p38 MAPK and ERK pathways, which are implicated in endogenous NO-induced apoptosis.

METHODS**Subject population**

Eighteen nonsmoking adults with clinically defined asthma were recruited from outpatient clinics. Asthma severity was assessed by means of an increase in asthma symptoms and increased bronchodilator requirements.²⁴ Patients with mild or moderate persistent asthma were enrolled in the study. Airflow limitation was present at the time of the laboratory visit in all patients with asthma, and there was a greater than 15% improvement in FEV₁ with inhaled albuterol. Patients did not receive corticosteroids, cromoglycate, theophylline, or other medications 6 weeks before presentation, except for rescue inhaled β_2 -agonists. Inhaled β -agonists were withheld for 12 hours before venipuncture.

Fourteen healthy nonsmoking subjects without evidence of allergic rhinitis or asthma as identified on the basis of history, negative serum test results for Phadiatop, and normal total IgE levels and eosinophil counts were recruited from our hospital staff or medical students. Spirometry and methacholine airway hyperresponsiveness test results were within normal limits in these subjects.

Preparation of eosinophils

One hundred milliliters of heparinized venous blood was obtained from each patient with asthma and healthy control subject. Granulocytes were separated from whole blood by using Ficoll-Hypaque density gradient centrifugation at 400g for 30 minutes, as previously described.¹⁰ The granulocyte pellet was washed twice and resuspended in RPMI-1640 containing 10% FCS. The contaminating RBCs were lysed by means of hypotonic shock. Eosinophils were isolated from granulocytes, as previously described.²⁵ Briefly,

leukocyte-rich suspension was carefully layered onto the multidiscontinuous Percoll density (polyvinylpyrrolidone-coated silica gel; 1.085, 1.090, and 1.100 g/mL; Pharmacia, Uppsala, Sweden) and then centrifuged at 700g for 20 minutes at 20°C. After centrifugation, cells were gently aspirated from each of the density gradient interfaces. The cells from each band were counted, and the purity of eosinophils was determined by means of examination of 200 cells on a Liu stain preparation. The recovery of eosinophils from gradients was more than 90%.

Expression of iNOS immunoreactivities in eosinophils

The iNOS expression in eosinophils was measured as previously described.²⁶ Briefly, eosinophils (1×10^6 cells/mL) were fixed and permeabilized with Ortho Permeafix (Becton Dickinson, Mountain View, Calif) for 20 minutes at room temperature. After 2 washes with PBS, the cells were incubated with 20 μ L of anti-iNOS rabbit polyclonal antibody or control IgG antibody (diluted 1:5 in PBS; Transduction Laboratories, Lexington, Ky) in the dark for 1 hour at 4°C. The cell pellet was labeled with 5 μ L of FITC-conjugated swine anti-rabbit IgG (Dakopatts, Glostrup, Denmark) and placed in the dark for 30 minutes at 4°C. Cells were analyzed by means of flow cytometry after 2 extensive washings with cold PBS containing 5% FCS.

Quantification of nitrite production by cultured eosinophils

The concentration of nitrite was measured as previously described.²⁶ Briefly, 50 μ L of the supernatant of culture medium was added to the purge vessel containing 5 mL of a reducing solution and 1% potassium iodide in acetic acid, which converts nitrite to NO. NO was then quantitated by using a specific chemiluminescence analyzer (NOA Model 280; Sievers, Boulder, Colo). Conversion of standard mixtures of nitrite and nitrate solutions to NO was 94% when compared with calibrated standards of NO gas. The level of nitrite in medium alone was subtracted from all the results of eosinophil cultures.

Assessment of apoptosis

To measure the apoptosis of eosinophils, cells were first fixed and permeabilized with ice-cold 70% alcohol at room temperature for 10 minutes. The cells were stained with 10 μ L of RNase A (100 μ g/mL; Sigma, St Louis, Mo) and 10 μ L of propidium iodide (40 μ g/mL, Sigma) and then incubated in a water bath at 37°C for 30 minutes. The proportion of cells within the hypodiploid DNA region was assessed by means of flow cytometry.²⁷ The morphologic change of eosinophil apoptosis was also confirmed by using a light microscope, including rounding up of nuclear contour, dense condensation of nuclear chromatin, and fragmentation of nuclear lobes.

DNA fragmentation survey

Two million purified eosinophils were lysed by means of incubation for 15 minutes in 400 μ L of cold mixture of 10 mmol/L Tris-HCl (pH 7.4), 0.2 mmol/L EDTA, and 0.2% Triton X-100. The lysate was centrifuged for 5 minutes at 10,000g. The supernatant was extracted with chloroform-isoamyl alcohol-phenol, and the aqueous phase was collected. The DNA was precipitated with 50% 2-propanol and 0.5 mol/L sodium acetate and left at -80°C overnight. After digestion with 50 μ g/mL RNase A (Boehringer Mannheim, Mannheim, Germany) for 1 hour at 37°C, the samples were electrophoresed through a 1.2% agarose gel and stained with 0.5 μ g/mL ethidium bromide.

Measurement of Bcl-2 expression

Cells were fixed and permeabilized with Ortho Permeafix (Becton Dickinson) for 20 minutes at room temperature to measure the

expression of intracellular Bcl-2. The cells were washed with PBS twice, followed by staining with anti-Bcl-2 mAb-conjugated FITC (Ancell, Bayport, Minn) for 30 minutes at 4°C. The cells were washed with ice-cold PBS twice and analyzed with a FACScan (Becton Dickinson). The FITC-labeled anti-IgG1 mAb (Ancell) was used as negative control.

Western blotting analysis of ERK and p38 kinase activities

Cells were lysed in a loading buffer after treatments. Protein samples were separated on 10% SDS-PAGE. The membranes were incubated with 0.1 mg/mL primary antibody for 2 hours at room temperature. Protein bands were transferred to a nitrocellulose membrane. Membrane was treated with a blocking solution at 37°C for 1 hour and at room temperature for 1 hour. Polyclonal anti-ERK2, monoclonal anti-phospho-ERK2, and monoclonal anti-phospho-p38 antibodies were used according to the manufacturer's recommendations. Cell extracts were subjected to SDS-PAGE and blotted onto nitrocellulose. Blots were developed by means of addition of peroxidase substrate, with enhancement by using ECL (Amersham, Arlington Heights, Ill) solution, and exposed to XAR films (Eastman Kodak, Rochester, NY).

Protocol

To study the effect of NO on cell apoptosis, eosinophils (1×10^6 cells/mL) were treated with PBS or N-nitro-L-arginine methyl ester (L-NAME, Sigma) at 1 and 10 mmol/L in the presence of 10% FCS and incubated with 5% CO₂ at 37°C for 16 hours. To evaluate the role of MAPK in the regulation of Bcl-2 expression, eosinophils were treated with an inhibitor of p38 MAPK (SB203580, 10 μ mol/L) or an inhibitor of p42/44 MAPK (PD98059, 30 μ mol/L). Freshly isolated and cultured cells were measured for Bcl-2 expression and apoptosis index. To study the effect of NO on p38 MAPK or ERK activities, eosinophils were treated with 1 mmol/L L-NAME for 10 minutes, and the activities of p42/44 and p38 MAPKs were measured. The p42/44 and p38 MAPK activities were abolished by PD98059 and SB203580, respectively, without any cross-reaction, indicating that the concentrations of these inhibitors used in this study were specific for both p42/44 and p38 MAPK.

Statistical analysis

Data are presented as means $\pm$ SEM. Student *t* tests were used to assess the significance of differences between groups. A *P* value of less than .05 was considered significant.

RESULTS

iNOS expression and nitrite generation in eosinophils

The magnitude of iNOS immunoreactivities on freshly isolated eosinophils was significantly higher in asthmatic patients (26.5 ± 3.6 mean fluorescence intensity [MFI], *n* = 8) than in control subjects (10.7 ± 0.9 MFI [*n* = 8], *P* < .01; Fig 1). Cultured eosinophils from asthmatic patients spontaneously released nitrite in the culture medium (2.6 ± 0.5 μ mol/L [*n* = 8]) to a greater extent than in healthy subjects (0.3 ± 0.1 μ mol/L [*n* = 8], *P* < .05; Fig 2). The spontaneous generation of nitrite by eosinophils from asthmatic patients was significantly inhibited by 1 mmol/L L-NAME (0.9 ± 0.2 μ mol/L [*n* = 8], *P* < .05; Fig 2), whereas nitrite generation by eosinophils of healthy subjects was not influenced (Fig 2).

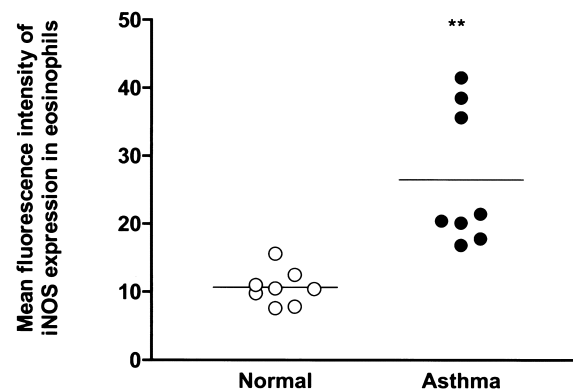


FIG 1. The individual MFI of iNOS of peripheral eosinophils from control subjects (*n* = 8, open circles) or patients with asthma (*n* = 8, filled circles). **P* < .01 compared with the healthy subject group.

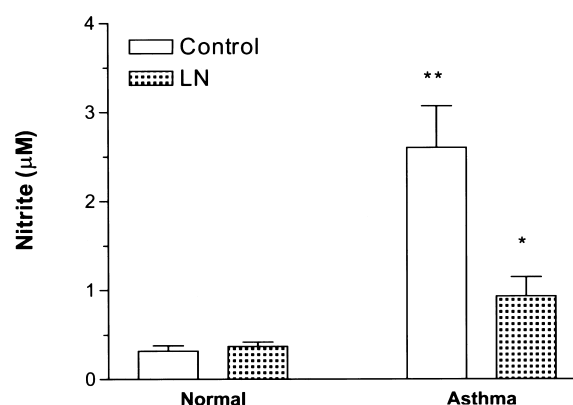


FIG 2. The spontaneous release of nitrite by cultured eosinophils from patients with asthma (*n* = 8, asthma) or healthy subjects (*n* = 8, normal) in the presence of 1 mmol/L L-NAME (hatched bars) or its control (open bars). ***P* < 0.01 compared with the control group of healthy subjects; **P* < .05 compared with the corresponding control group. Values are presented as means $\pm$ SEM.

Effect of NO on eosinophil apoptosis

Eosinophil apoptosis is expressed as the apoptosis index (ie, the number of apoptotic cells/total number of cells). The spontaneous apoptosis index of eosinophils in healthy subjects after 16 hours' culture increased by 14.5 ± 6.8 -fold (compared with the apoptotic index of freshly isolated eosinophils), which was a greater extent than that seen in asthmatic patients (1.7 ± 1.2 -fold, *P* < .05). In asthmatic patients inhibition of endogenous NO by means of L-NAME decreased eosinophil apoptosis dose dependently ($9.9\% \pm 1.4\%$ at 1 mmol/L, *P* < .05; $8.5\% \pm 1.9\%$ at 10 mmol/L, *P* < .01, *n* = 8) compared with that seen in control subjects ($12.7\% \pm 2.1\%$, Fig 3). In healthy subjects eosinophil apoptosis was significantly attenuated by L-NAME only at the higher concentration (10 mmol/L, Fig 3). Eosinophil apoptosis in asthmatic patients was also confirmed by means of Agarose gel electrophoresis of DNA (Fig 4). The attenuation of laddering DNA extracts by means of L-NAME was evident (Fig 4).

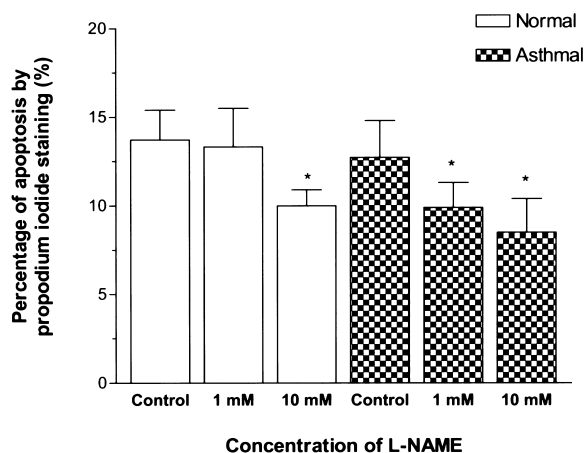


FIG 3. Effect of L-NAME at various concentrations on the apoptosis of eosinophils from patients with asthma ($n = 8$, filled bar) and healthy subjects ($n = 8$, open bar). Data are presented as means $\pm$ SEM. * $P < .05$ compared with the control of corresponding group.

Bcl-2 expression in eosinophils

Bcl-2 expression was higher in freshly purified eosinophils from asthmatic patients (192.5 ± 51.3 MFI [$n = 8$]) compared with that seen in healthy subjects (11.9 ± 2.3 MFI [$n = 8$], $P < .01$; Fig 5). The iNOS level was inversely related to Bcl-2 expression in eosinophils of asthmatic patients ($r = -0.728$ [$n = 8$], $P < .05$). After incubation for 16 hours, the Bcl-2 expression of eosinophils was increased by L-NAME dose dependently in asthmatic patients at 1 mmol/L (82.5 ± 7.6 MFI [$n = 8$], $P < .05$) and 10 mmol/L (128.3 ± 33.3 MFI [$n = 8$], $P < .05$) compared with that seen in control subjects (60.1 ± 7.3 MFI, $n = 8$; Fig 6). In healthy subjects the Bcl-2 expression was significantly increased by L-NAME only at 10 mmol/L (64.2 ± 11.1 MFI, $n = 8$, $P < .05$) compared with that seen in healthy subjects (37.8 ± 4.6 MFI, $n = 8$; Fig 6).

MAPK pathway implicated in Bcl-2 expression and eosinophil apoptosis

The baseline activities of phosphorylated ERK and p38 MAPK were higher in the eosinophils of asthmatic patients ($n = 3$) than those of healthy subjects ($n = 3$, Fig 7). L-NAME (1 mmol/L) enhanced the activities of phosphorylated ERK and phosphorylated p38 MAPK in asthmatic patients (Fig 7). Either ERK inhibitors (SB203580, 10 μ mol/L) or p38 MAPK (PD98059, 30 μ mol/L) significantly reduced Bcl-2 expression in the eosinophils of asthmatic patients (40.0 ± 10.1 MFI [$n = 8$], $P < .05$, and 33.8 ± 6.8 MFI [$n = 8$], $P < .05$, respectively) compared with that seen in healthy subjects (73.0 ± 13.6 MFI [$n = 8$]) (Fig 8). Eosinophil apoptosis was significantly increased after an inhibition of ERK ($16.4\% \pm 1.5\%$ [$n = 8$], $P < .01$) or p38 MAPK ($15.6\% \pm 1.5\%$ [$n = 8$], $P < .01$) in asthmatic patients compared with that seen in healthy subjects ($13.3\% \pm 1.3\%$, $n = 8$; Fig 9). However,



FIG 4. Electrophoresis of DNA extracted from eosinophils of a healthy subject (lane 1) and an asthmatic patient (lanes 2 and 3) after culture for 16 hours with medium alone (lanes 1 and 2) or 1 mmol/L L-NAME (lane 3).

neither ERK nor p38 MAPK inhibitors affected Bcl-2 expression or eosinophil apoptosis in healthy subjects (Figs 8 and 9).

DISCUSSION

In the present study we have shown that eosinophils from asthmatic patients survived longer than those of healthy control subjects. Our results are consistent with a previous report²⁸ and indicate that prolonged eosinophil survival or reduced apoptosis might play a central role in the maintenance of chronic persistent airway inflammation in asthma. We also demonstrated that iNOS activity and endogenous NO production were upregulated in eosinophils of patients with asthma. Inhibition of

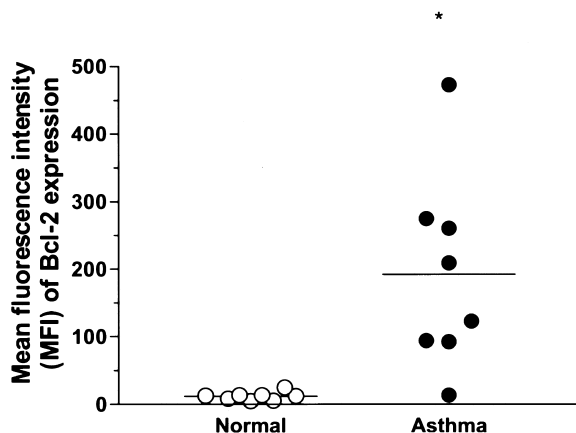


FIG 5. The individual MFI of Bcl-2 protein expression in freshly isolated eosinophils of healthy subjects ($n = 8$, open circles) or asthmatic patients ($n = 8$, filled circles). * $P < .05$ compared with the healthy subject group.

endogenous NO production by L-NAME dose dependently reduced eosinophil apoptosis in asthmatic patients, indicating that enhanced endogenous NO production by eosinophils is implicated in the regulation of eosinophil apoptosis in asthma. NO has a wide array of potentially important proinflammatory effects in asthma.²⁹ There is increasing evidence showing that NO represents a key regulator of cell survival.³⁰

Battinelli and Loscalzo³¹ reported that NO-induced apoptosis in megakaryocytic cell lines might be related to the toxic effect of peroxynitrite formation. NO-regulated apoptosis also has been shown to result from the activation of many apoptotic effectors, including p53,³² stress-activated protein kinase,³³ the Bcl-2 family of proteins,³⁴ and cytochrome C.³⁵ The Bcl-2 family are vital regulators of programmed cell death in numerous systems. Bcl-2 family proteins regulate apoptosis, including antiapoptotic proteins, such as Bcl-2 and Bcl-x, and proapoptotic proteins, such as Bax and Bak.¹⁵ NO exposure induces nuclear phosphoprotein p53 expression,³² which induces cell apoptosis through altering the balance of the anti-apoptosis and proapoptosis gene products Bcl-2 and Bax.³⁶ Here we have demonstrated that inhibition of NO production significantly upregulates Bcl-2 expression, suggesting that attenuation of Bcl-2 expression is contributory to NO-induced eosinophil apoptosis in asthmatic patients. However, NO is not the only mediator to attenuate Bcl-2 to induce eosinophil apoptosis. An increase in the intracellular cyclic adenosine monophosphate levels was also reported to contribute to eosinophil apoptosis with suppression of Bcl-2 expression.³⁷ In addition, GM-CSF, which is antiapoptotic to eosinophils, can induce NO production³⁸ without influence on Bcl-2 activity.³⁹

In the present report Bcl-2 expression was upregulated in the eosinophils of asthmatic patients compared with that seen in healthy subjects. Increased numbers of Bcl-2-positive eosinophils in the sputum of asthmatic patients has been shown to be correlated with asthma

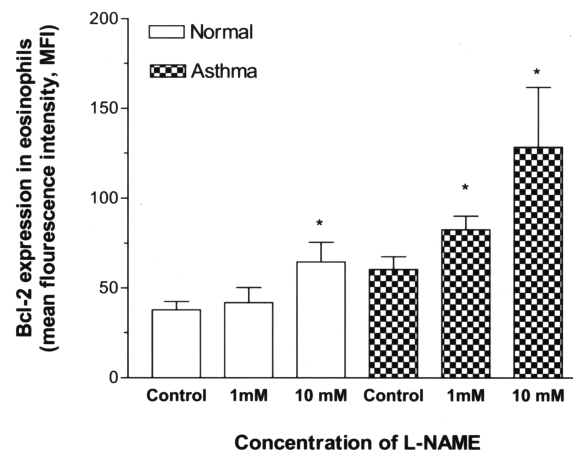


FIG 6. The MFI of Bcl-2 expression in cells treated with L-NAME or with medium only (control) for 16 hours in patients with asthma ($n = 8$, filled bars) or in healthy subjects ($n = 8$, open bars). * $P < .05$ compared with the corresponding control. Data are presented as means $\pm$ SEM.

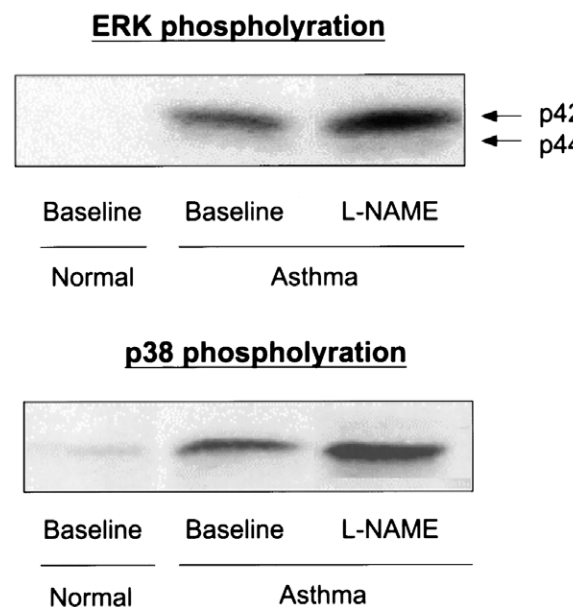


FIG 7. Western blot analysis of p38 or ERK phosphorylation. p38 or ERK phosphorylation was increased when eosinophils were cultured with L-NAME compared with culture in medium alone.

inflammation,⁴⁰ suggesting that Bcl-2 expression is important in the maintenance of persistent eosinophilic inflammation in asthma. The Bcl-2 protein appears to be expressed constitutively in freshly isolated eosinophils and is augmented by IL-5 stimulation.³⁷ Apoptosis is reduced in eosinophils cultured in the presence of IL-5 for 24 hours accompanied by upregulation of Bcl-2 mRNA and protein expression without detectable changes in Bax or Bcl-x.^{41,42} Although the exact mechanism for upregulated Bcl-2 expression in asthmatic eosinophils was not elucidated in the present study, possibly it can be attributed to increased IL-5 level in asth-

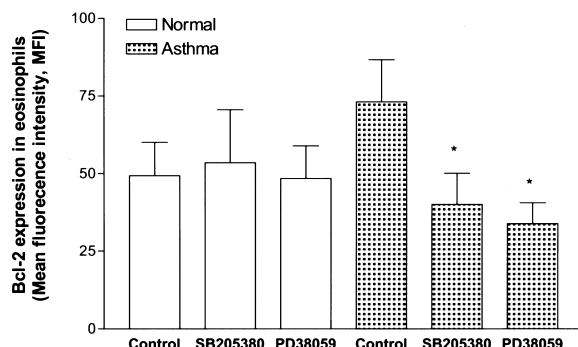


FIG 8. The MFI of Bcl-2 expression in eosinophils of asthmatic patients ($n = 8$, filled bar) and healthy subjects ($n = 8$, open bar) in the presence of SB203580 (10 $\mu\text{mol/L}$) or PD98059 (30 $\mu\text{mol/L}$) or medium alone (control) for 16 hours. * $P < .05$ compared with the corresponding control. Data are presented as means $\pm$ SEM.

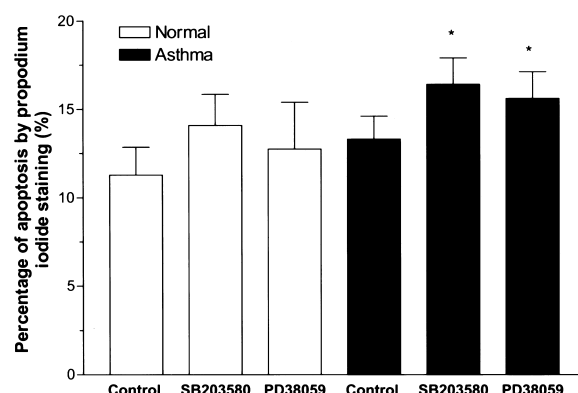


FIG 9. Effect of SB203580 (10 $\mu\text{mol/L}$) or PD98059 (30 $\mu\text{mol/L}$) on eosinophil apoptosis from asthmatic patients ($n = 8$, filled bar) and healthy subjects ($n = 8$, open bar). Data are presented as means $\pm$ SEM. * $P < .01$ compared with the corresponding control.

ma, as shown in the previous report that IL-5 levels in sputum were correlated with Bcl-2 protein expression in eosinophils.⁴⁰ Thus we might presume that IL-5 might upregulate Bcl-2 expression through modulation of NO synthase. However, in an animal study, anti-IL-5 antibody reduces NO production of eosinophils.⁴³ Future studies to investigate the relationship between IL-5 and NO synthase might be needed.

Activation of ERK and p38 MAPKs is implicated in the regulation of eosinophil differentiation, cytokine production, and degranulation.²¹ Inhibition of both signal transduction pathways reduces eosinophil survival.⁴⁴ ERK1/2 was shown to regulate protein synthesis of Bcl-2. Inhibition of ERK1/2 activities caused a downregulation of antiapoptotic homolog Bcl-2, Mcl-1, and Bcl-X(L) expression without affecting the proapoptotic levels of Bax and Bak in pancreatic cancer cells.⁴⁵ Administration of the p38 MAPK inhibitor SB239063 in guinea pigs markedly reduced ovalbumin-induced pulmonary eosinophil influx and increased apoptosis of eosinophils retrieved from bronchoalveolar lavage fluid and cultured

in the presence of IL-5.⁴⁴ Thus ERK1/2 and p38 MAPK activities are closely associated to the apoptotic signaling pathway of eosinophils.⁴⁶ Our results have shown that inhibition of p38 MAPK and ERK1/2 significantly decreases Bcl-2 expression in eosinophils of asthmatic patients and subsequently increased eosinophil apoptosis, indicating that p38 MAPK and ERK1/2 were the upstream signaling pathways regulating Bcl-2 expression.

The MAPK signaling pathway is vulnerable to NO.⁴⁶ NO donors or cyclic guanosine monophosphate analogs attenuate mechanical strain-induced activation of p38 MAPK in mesangial cells.⁴⁷ Endogenous NO inhibits cellular proliferation in response to endothelin 1 through suppression of the MAPK cascade.⁴⁸ Our results also showed that the inhibition of endogenous NO enhances the activities of p38 MAPK and ERK1/2 in the eosinophils of patients with asthma, which in turn contributes to Bcl-2 upregulation in the eosinophils of patients with asthma. Taken together, NO might directly interfere with MAPK activities to regulate eosinophil survival.

We conclude that Bcl-2 upregulation is contributory to prolonged survival of eosinophils in patients with asthma. Bcl-2 expression of eosinophils in asthmatic patients is regulated through the p38 MAPK and ERK signaling pathways. Inhibition of endogenous NO increases Bcl-2 expression in eosinophils from asthmatic patients through ERK or p38 MAPK and then decreases the apoptosis. The concomitant increase in iNOS activity and endogenous NO production by asthmatic eosinophils could play a self-limiting role in the regulation of apoptosis.

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