

Original Article

Expression of the Apoptosis Inhibitor Bcl-2 in Sputum Eosinophils in Patients with Asthma

Ghasem Azimi, Hesam Amini , Niosha Andalibi

Dept. of Internal Medicine, Shahed University, Tehran, Iran

ABSTRACT

Background and Objectives: Apoptosis of eosinophils is of significant value in assessing the airway inflammation in patients with asthma. Our purpose was to investigate the degree of expression of the Bcl-2 protein in sputum eosinophils during acute asthma exacerbation and its relationship with exacerbation severity.

Materials and Methods: The study was carried out in Mostafa Khomeini Hospital, Tehran, Iran in March 2008. Sputum was obtained from 15 asthmatic patients and 13 healthy subjects as a control group. Number of eosinophils was counted and Bcl-2+ eosinophils were stained using immunocytochemistry.

Results: Sputum eosinophils and Bcl-2+ eosinophils were significantly higher in patients with acute exacerbation than controls ($P<0.05$).

Conclusion: Bcl-2 prolongs survival and decreases apoptosis of airway eosinophils during acute asthma exacerbation. Eosinophil apoptosis and inhibition of Bcl-2 represent a target for new and effective therapeutic strategies of asthma.

Keywords: Apoptosis, Asthma, B-bcl-2 Proteins, Eosinophil, Sputum

Introduction

Asthma is one of the most common causes of referring to the pulmonary clinics and is characterized by reversible airway obstruction. Eosinophils are known to play a pivotal role in asthmatic airway inflammation. Infiltration of eosinophils into the bronchial wall and respiratory epithelial damage are two distinctive features of asthma (1,2). These bronchial changes involve four steps namely, enhanced eosinophil production,

recruitment of lung tissue, activation of eosinophils, and release of mediators. The prolongation of eosinophil survival is important in the pathogenesis of asthmatic airway inflammation. Apoptosis plays a central part in normal tissue homeostasis as well as having a role in various clinical diseases characterized by either increased or decreased cell survival (3). It is thought to be critically important in promoting the clearance of inflammatory cells and the resolution of inflammation. Apoptosis of eosinophils may be

Received: 28 January 2009

Accepted: 13 August 2009

Address communications to: Dr Ghasem Azimi, Department of Internal Medicine, Mostafa Khomeini Hospital, Shahed University, Tehran, Iran

Email: gh.Azimi @ yahoo.com

clinically relevant in asthma, promoting the removal of airway eosinophils and contributing to clinical improvement. Among apoptosis suppressing genes, bcl-2 prevents apoptosis either through altering cell cycle rates or by activating antioxidant-associated mechanisms (4, 5).

Apoptosis is characterized by chromatin aggregation; DNA fracture and exploring the cell walls morphologically. Three gene groups play a significant role in apoptosis:

Bcl2 apoptosis inhibitory genes (B cell lymphoma/Leukemia) (6)

Apoptosis stimulatory genes such as Caspase (7)

Intermediate genes such as Fas & Fasligand & P53(8, 9)

Bcl2 is an internal protein inhibits apoptosis throughout the different ways.

This study was undertaken to determine whether bcl-2 expression in sputum reflected the clinical severity of patients with asthma comparing to the healthy controls.

Materials and Methods

Twenty- eight subjects for this case control study were selected including 15 patients and 13 healthy controls. The patients with asthma were recruited for this study from the Division of Allergy, Department of Internal Medicine, Mostafa Khomeini Hospital Tehran, Iran, (we took the patients agreement to participate in this study). The diagnoses of asthma were established in the patients according to their symptoms of recurrent episodic wheezing and dyspnea and they were included of 7 men and 8 women (46%, & 54%, respectively).

Healthy controls were recruited from staff at the Shahed University of Medical Sciences. They had past medical and family history of allergy, asthma, and atopia.

Sputum induction

All subjects were premeditated with inhaled salbutamol 2 puffs (200 mg). Subjects inhaled hypertonic saline solution aerosols generated by a nebulizer for 10 minutes. They were encouraged to cough deeply and frequently during hypertonic saline inhalation. Most of the patients produced adequate Sputum (7 ml and more) during the first 10 minutes (10).

Sputum processing

The method of sputum examination was done as reported earlier (11). Sputum was treated by adding equal volumes of 0.1% dithiothreitol followed by equal volumes of Dulbecco's phosphate-buffered saline (D-PBS). The sample was then mixed gently and placed in 37°C for 15 min on hot plates to ensure complete homogenization, and subsequently was centrifuged at 1500 rpm for 10 min. The upper liquid emptied and the plate was distributed in 3 slides. Two slides were dried by air- drying method and fixed by formaldehyde 4% for 30 min to immunocytochemistry investigations. The third slide was stained for cell count by Wright Geimsa. Assessing and investigations were done by laboratory experts.

Immunocytochemistry Analysis of BCL-2

Immunocytochemical analysis was performed on sputum slides. The primary antibody was applied for 15 min with anti bcl-2 antibody (Dako, Denmark) analysis was performed by peroxidase reaction and then bcl-2 exposed to the Teriton 3x-100% including goat serum 10% for 30 min in room temperature and then incubated with primary antibody bcl-2 enzyme 1/300 for about 72 h in 4°C. The samples were incubated with secondary antibody, peroxides conjugated, 1/100 (DAKO, Germany) for 2 h in 37° C after washing with buffer saline. Daminobenzidin (DAB) 0.02% with H₂O₂ 0.015% and nickel sulfite 0.4% in buffer saline were added to form the brown sedimentation due to DAB/Peroxidase and after that samples were dried, cleaned and were assessed by ZEISS microscope. Negative control was done to determine antibody reaction and for this reason, we omitted primary antibody in protocol. The cells that stained for bcl-2 were counted. Dates were calculated in form of mean and SD and t test was done to compare between two groups.

Results

The results of the investigation showed that the eosinophils, which were dyed by Wright Geimsa, were seen in bluish color and the number in patients was more than control group.

We counted eosinophils, which were brown because of immunity reaction with anti-bcl-2 .The results showed percentage of bcl-2+ eosinophils in sputum was more in patients with acute asthma comparing to the control (Table 1).

Table 1: Mean and Sd for eosinophils in study and control groups

Groups	Mean & Sd of eosinophils	Percentage of eosinophils with bcl-2 positive
Study or Asthmatic patients	2.0 ± 16.1*	25.4**
Control	1.8 ± 0.5	0.2

* indicate significant difference relative to control) $P \leq 0.05$)

** indicate significant difference relative to control ($P \leq 0.05$)

Discussion

Eosinophils play an important role in patients with asthma. Inhibition of eosinophil apoptosis leads to asthma exacerbation. Bcl-2 prevents apoptosis through the antioxidants mechanisms. Related reports showed bcl-2 increasing in bronchial biopsy of asthmatic patients. A number of studies were done on sputum eosinophils apoptosis. Some of them showed increasing bcl-2 apoptosis in macrophages and lymphocytes comparing to the controls and found that it is related to the asthma severity (4). The others showed eosinophils in sputum during the acute asthma exacerbation and 2 weeks after treatment (12). They realized high increased number of sputum eosinophils during attacks and decreased number of sputum eosinophils after treatment. IL5 and expression of bcl2 is correlated with asthma severity and IL5 will increase the eosinophils survival (13). Moreover, eosinophils culture with IL 5 in vitro showed apoptosis inhibition and induced MRANA bcl-2 and Protein expression as well (14). Thus IL5 has an inhibitory effect on the eosinophils apoptosis by regulating the bcl-2 expression (15).

Hasala *et al.* reported the effect of the levocetirizine on inflammatory mediators including cytokines, growth factors, proteinases, and antiproteinases produced by eosinophils, which may be of importance in allergic inflammation and airway remodeling (16).

The current study showed that sputum eosinophil percentage in patients with acute asthma is more than controls, which is confirmed by previous studies. It is also determined that bcl-2 sputum eosinophils in patients with acute asthma are more than controls.

Conclusion

There was a significant positive correlation between number of bcl-2 sputum eosinophils and sputum eosinophils percentage. In summary, percentage of

eosinophils and bcl-2 sputum eosinophils in patients with acute asthma is more than controls. As a result, bcl-2 may increase the survival of eosinophils and decrease apoptosis in airway eosinophils with acute asthma and causes airway inflammation.

Acknowledgements

This research has been supported financially by Shahed University. In addition, the authors sincerely appreciate Dr. Mohamad Reza Jalali for technical assistance in Mostafa Khomeini Hospital Pathology Laboratory

References

1. Spinozzi F, de BD, de Benedictis FM. Apoptosis, airway inflammation and anti-asthma therapy: from immunobiology to clinical application. *Pediatr Allergy Immunol* 2008;19(4):287-95.
2. Kankaanranta H, Lindsay MA, Giembycz MA, Zhang X, Moilanen E, Barnes PJ. Delayed eosinophil apoptosis in asthma. *J Allergy Clin Immunol* 2000;106(1 Pt 1):77-83.
3. Ryttila P, Metso T, Petays T, Sohlman A, Tyolahti H, Kohonen-Jalonen P, *et al.* Eosinophilic airway inflammation as an underlying mechanism of undiagnosed prolonged cough in primary healthcare patients. *Respir Med* 2002; 96(1):52-8.
4. Vignola AM, Chiappara G, Gagliardo R, Gjomarkaj M, Merendino A, Siena L, *et al.* Apoptosis and airway inflammation in asthma. *Apoptosis* 2000;5(5):473-85.
5. Vignola AM, La GS, Chiappara G, Benkeder A, Bellia V, Bonsignore G. Cellular network in airways inflammation and remodelling. *Paediatr Respir Rev* 2002;3(1):41-6.
6. Craig RW. The bcl-2 gene family. *Semin Cancer Biol* 1995;6(1):35-43.
7. Kumar S. ICE-like proteases in apoptosis. *Trends Biochem Sci* 1995;20(5):198-202.

8. Matsumoto K, Schleimer RP, Saito H, Iikura Y, Bochner BS. Induction of apoptosis in human eosinophils by anti-Fas antibody treatment in vitro. *Blood* 1995;15;86(4):1437-43.
9. Behrmann I, Walczak H, Krammer PH. Structure of the human APO-1 gene. *Eur J Immunol* 1994;24(12):3057-62.
10. Fahy JV, Liu J, Wong H, Boushey HA. Cellular and biochemical analysis of induced sputum from asthmatic and from healthy subjects. *Am Rev Respir Dis* 1993;147(5):1126-31.
11. Frigas E, Gleich GJ. The eosinophil and the pathophysiology of asthma. *J Allergy Clin Immunol* 1986;77(4):527-37.
12. Woolley KL, Gibson PG, Carty K, Wilson AJ, Twaddell SH, Woolley MJ. Eosinophil apoptosis and the resolution of airway inflammation in asthma. *Am J Respir Crit Care Med* 1996;154(1):237-43.
13. Jang AS, Choi IS, Lee S, Seo JP, Yang SW, Park CS. Bcl-2 expression in sputum eosinophils in patients with acute asthma. *Thorax* 2000;55(5):370-4.
14. Dewson G, Walsh GM, Wardlaw AJ. Expression of Bcl-2 and its homologues in human eosinophils. Modulation by interleukin-5. *Am J Respir Cell Mol Biol* 1999;20(4):720-8.
15. Ochiai K, Kagami M, Matsumura R, Tomioka H. IL-5 but not interferon-gamma (IFN-gamma) inhibits eosinophil apoptosis by up-regulation of bcl-2 expression. *Clin Exp Immunol* 1997;107(1):198-204.
16. Hasala H, Janka-Junttila M, Moilanen E, Kankaanranta H. Levocetirizine and cytokine production and apoptosis of human eosinophils. *Allergy Asthma Proc* 2007;28(5):582-91.