In vitro arsenic trioxide induces apoptosis in T cells of asthmatic patients by a Bcl-2 related mechanism

QIN Dong-yun[1], HUANG Ren[2*], WU Tie[1]

(*1. Department of Pharmacology, Guangdong Medical College, Zhanjiang 524023, China;*
2. Guangdong Experimental Animal Monitoring Department, Guangzhou 510260, China)

Abstract: This study examined the effects of arsenic trioxide on apoptosis and interleukin-4 release in T cells of asthmatic patients *in vitro* and investigated the role of Bcl-2 in the active mechanism. T cells were isolated from asthmatic patients (n = 21) and healthy controls (n = 20), and then treated with arsenic trioxide and dexamethasone. Cell apoptosis was measured using fluorescence microscopy, flow cytometry and a cytochrome c ELISA kit. Interleukin-4 levels in the serum and in supernatants from T cells were quantified by ELISA. Flow cytometric analysis and immunofluorescence studies were performed to determine Bcl-2 expression. T cells of the asthmatic patients (*i. e.* without treatment) exhibited decelerated spontaneous apoptosis after 24 h incubation *in vitro* when compared to T cells of the healthy controls. With dexamethasone treatment, an increase in apoptosis of T cells was not significantly different between both groups, irrespective of the method used. Arsenic trioxide treatment, however, significantly increased the apoptosis of T cells of the asthmatic group and showed a slight effect on the control group. In asthmatic patients, elevated levels of interleukin-4 and up-regulated Bcl-2 expression were detected. Moreover, *in vitro*, T cells of asthmatic patients spontaneously released more interleukin-4 and exhibited more Bcl-2 expression than T cells from the control group. Arsenic trioxide treatment significantly decreased interleukin-4 release and down-regulated Bcl-2 expression in asthmatic patients, while it only slightly affected healthy controls. Dexamethasone treatment decreased interleukin-4 release in both groups examined. It did not significantly influence Bcl-2 expression. These results suggest that arsenic trioxide induces T cell apoptosis and decreases interleukin-4 release in T cells of asthmatic patients *in vitro* and that down-regulation of Bcl-2 expression may be an important mechanism.

Key words: arsenic trioxide; T cell; apoptosis; interleukin-4; asthma; Bcl-2
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三氧化二砷通过 Bcl-2 相关机制诱导哮喘患者 T 细胞凋亡

覃冬云[1], 黄 韧[2*], 吴 铁[1]

(1. 广东医学院 药理学教研室, 广东 湛江 524023; 2. 广东省实验动物监测所, 广东 广州 510260)

摘要: 本研究观察了三氧化二砷对哮喘患者 T 细胞凋亡、白细胞介素-4 分泌的影响,并探讨了 Bcl-2 的作用。分离哮喘患者(n = 21)和健康对照者(n = 20)的外周血 T 细胞,分别加入三氧化二砷和地塞米松培养 24 h。用荧光显微术、流式细胞仪 DNA 含量分析法和细胞色素 c ELISA 试剂盒检测 T 细胞凋亡,用 ELISA 的方法测量血清和细胞培养上清液白细胞介素-4 水平,用免疫荧光流式细胞分析法测定 Bcl-2 基因表达。与健康对照者比较,哮喘患者 T 细胞体外培养 24 h 后自发凋亡减慢。地塞米松使哮喘患者和健康对照者的 T 细胞凋亡率均增加,两试验组间增加幅度无显著差异。三氧化二砷显著增加哮喘患者 T 细胞凋亡率,但对健康对照者 T 细胞凋亡影响不明显。哮喘患者

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* Corresponding author Tel: 86 - 20 - 34063369, Fax: 86 - 20 - 34063761, E-mail: huangren2217807@126.com

血清白细胞介素-4 水平升高,T 细胞 Bcl-2 表达上调。而且,在体外培养 24 h 后,哮喘患者 T 细胞比健康对照者 T 细胞自发分泌的白细胞介素-4 及 Bcl-2 的表达水平均有所提高。三氧化二砷显著减少哮喘患者 T 细胞白细胞介素-4 分泌,下调 Bcl-2 表达,但对健康对照者 T 细胞无明显影响。地塞米松可使两试验组 T 细胞释放白细胞介素-4 显著减少,但对两试验组 T 细胞 Bcl-2 基因表达影响不明显。结果提示:三氧化二砷在体外可诱导哮喘患者 T 细胞凋亡,减少白细胞介素-4 分泌,下调 Bcl-2 基因表达可能是其重要机制之一。

关键词: 三氧化二砷; T 细胞; 细胞凋亡; 白细胞介素-4; 哮喘; Bcl-2

Chinese traditional medicine treats asthma patients with a mineral drug containing arsenic, and with this drug, special curative effects are achieved[1]. In the Song Dynasty in China, Zijindan is clinically and frequently used to treat asthma, with more than 90% of the patients safety controlled asthmatic attacks[2]. The main component of Zijindan is arsenic trioxide. Our previous research indicated that mice with allergic asthma could be treated effectively with arsenic trioxide[3]. Arsenic trioxide improved both the lung function of the mice and suppressed infiltration of inflammatory cells into the airways. The exact mechanism behind this positive effect remains uncertain.

T cells have a central regulatory role in the pathogenesis of asthma. Activated T cells secret Th2-like cytokines such as interleukin (IL)-4, which stimulates bronchoalveolar lavage fluid eosinophilia, hyperreactivity (AHR) and goblet cell hyperplasia[4]. T cell apoptosis is delayed in asthma, which may be the cause of the persistent infiltration of allergic inflammatory cells into the airways. Inducing T cell apoptosis leads to depression of T cell proliferation and to suppression of the immune response.

Arsenic trioxide is considered one of the most orthodox anti-tumor drugs in China. It has been confirmed that arsenic trioxide can cure acute promyelocytic leukemia and other malignant tumors. *In vitro* studies show that the mechanism involves the suppression of cell proliferation and the induction of cell apoptosis[5]. Arsenic trioxide has been shown to induce apoptosis in a variety of blood cells by activation of the mitochondrial pathway of cell death[6]. The mitochondrial apoptotic pathway is regulated by apoptosis-related proteins (*e. g.* Bcl-2) as well as cytokines (*e. g.* IL-4) [7]. Therefore, we investigated whether the mechanism behind arsenic trioxide treatment for asthma is related to an induction of T cell apoptosis and a subsequent decrease in IL-4 release. The aim of this study is to examine the effects of arsenic trioxide on peri-blood T cell apoptosis and IL-4 release *in vitro*, in cells obtained from asthmatic patients and normal healthy controls, and also try to investigate the mechanism involved in arsenic trioxide-induced apoptosis of T cells, such as the role of Bcl-2 expression.

Materials and methods

Subjects Approval for this study was granted by the Clinical Ethics Committee and informed consent was obtained from each subject. Patients with an acute attack of asthma (n = 21) were examined, with the mean age of patients at (48.9 ± 12.8) years. The diagnosis was based on clinical history, clinical examination, and laboratory findings and was consistent with diagnostic criteria outlined at the National Asthma Conference (1997). All patients were defined as acute attack asthmatics with AHR. Lower maximal expiratory flows were at 25% and 50% of vital capacity (MEF25, MEF50), combined with conditions of expiratory dyspnea and wheezing. None of the examined patients had any clinical signs of infection such as fever or neutrophilic leukocytosis. None had received blood transfusions or was under treatment with steroids or immunosuppressive therapy for at least one week prior to the study, and there were no signs of acute respiratory failure or autoimmune disease in any subjects at the time of study entry. The control group consisted of 20 healthy age-matched and sex-matched university workers who had undergone an annual health examination. Examinations confirmed them to be free of major cardiopulmonary and autoimmune diseases. Table 1 characterizes the study subjects.

Isolation of peripheral blood T lymphocytes The separation of T cells from blood was performed using the lymphoprep and nylon wool column method[8]. In short, T cells were washed twice with RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), suspended in RPMI 1640 medium containing 10% FCS, 100 $\mu g \cdot L^{-1}$ streptomycin and 1×10^5 $u \cdot L^{-1}$ penicillin at a density of 5×10^8 cells $\cdot L^{-1}$. Cell viability was determined using Trypan Blue exclusion, with results indicating a cell population viability of greater than 98%. T cells

($CD3^+$ cells) represented 92% of the cells (determined by flow cytometric analysis and indirect immunofluorescence techniques).

Treatment of T lymphocytes with arsenic trioxide and dexamethasone T cell suspensions were distributed into 24-well plastic plates (Falcon, Bedford, MA, USA), 5×10^5 cells per well, and treated with 0.1 $\mu g \cdot mL^{-1}$ arsenic trioxide (Sigma, USA) and 5 $\mu g \cdot mL^{-1}$ dexamethasone. Cells not treated with the chemical additives were labeled as "spontaneous release" for the purposes of this study. Samples were incubated for 24 h in a humidified atmosphere at 37 ℃, 5% CO_2. After incubation, cell culture supernatants were collected for assay of IL-4, centrifuged and frozen immediately at −70 ℃, and stored for no longer than 3 weeks. The cells were used for apoptosis measurements or for assay of Bcl-2 expression.

IL-4 assay Human IL-4 levels in the serum and in supernatants obtained from T cells were quantified by ELISA, using a commercially available kit (Endogen, Woburn, MA, USA). The kits employed a specific monoclonal antibody immobilized on a 96-well microtiter plate that bound IL-4 in the aliquot and a second enzyme-conjugated specific polyclonal antibody. Following several washings in order to remove unbound substances and antibodies, a substrate solution was added to the wells. Color development was stopped with the addition of sulphuric acid and the intensity of color was measured using a microtiter plate reader (E-max, Molecular Devices Co, Menlo Park, CA, USA) at 450 nm (correction at 550 or 540 nm). The detection limit was 3 $pg \cdot mL^{-1}$. Intra-assay variations were less than 6%.

Apoptosis measurement After incubation with arsenic trioxide and dexamethasone, the cells were centrifuged and suspended in 50 μL of RPMI 1640. A 25 μL aliquot of cells was stained with a 1 : 1 stock solution of 100 $\mu g \cdot mL^{-1}$ acridine orange plus 100 $\mu g \cdot mL^{-1}$ of ethidium bromide in PBS[9]. After 2 min, the suspension was placed on a microscope slide and covered with a coverslip. A minimum of 500 cells was scored under the fluorescence microscope and categorized as normal, apoptotic, or necrotic. "Healthy" cells had a normal green nucleus, and apoptotic cells had condensed or fragmented bright green chromatin. Late apoptotic cells with secondary necrosis displayed condensed or fragmented orange chromatin[9]. Both apoptotic types of cells were included in the "apoptotic category". The results were expressed as the percentage of apoptotic cells.

Another 25 μL aliquot of cells was stained and fixed with propidium iodide. DNA content analysis was performed using flow cytometry to detect the rate of apoptosis. The apoptosis mark is the DNA subdiploid-peak.

Cell apoptosis was also measured by cytochrome c release from mitochondria using a Cytochrome c ELISA kit from Bender Medsystem Diagnostic GmbH (Vienna, Austria). The assay was performed according to the manufacturer's instruction. After incubation as described above, cells were centrifuged, washed in PBS, re-suspended in Lydid Buffer and incubated for 1 h at room temperature. After centrifugation ($1\,000 \times g$ for 15 min), the cytochrome c concentration in the cytosol was measured. Samples were transferred into wells coated with a monoclonal antibody specific for cytochrome c, washed, and incubated with a biotinylated second antibody. Following incubation and washing, a streptavidine-HRP complex was added. After washing, a substrate solution was added to the wells. Colour development was stopped with sulphuric acid and the intensity of color was measured at 450 nm (correction at 610 nm). The limit of cytochrome c detection was 0.08 $ng \cdot mL^{-1}$.

Bcl-2 expression assay and flow cytometric analysis After incubation with arsenic trioxide and dexamethasone, as described above, the cells were centrifuged and washed twice in PBS containing 1% bovine serum albumin. Immunofluorescence studies were performed, using a combination of fluorescein isothyocyanate (FITC) conjugated mouse monoclonal antibodies: Bcl-2-FITC from R&D System, MN, USA.

Cells (5×10^5) were added to a flow cytometry tube and stained with monoclonal antibodies against cell surface markers (Bcl-2-FITC) for 20 min at room temperature and analyzed by flow cytometry directly after preparation.

The cells were collected using a FACSCalibur flow cytometer equipped with 488 nm argon laser (Becton Dickinson) and analyzed with CellQuest Software. 10 000 total events were collected. An isotype-matched negative control was used in the assay to distinguish positive and negative results.

Statistical analysis Values are expressed as $\bar{x} \pm s$. The significance of differences was determined with the Manny-Whitney u test for comparisons between two groups (control and experimental one), the

ANOVA Friedmans' test for multiple comparisons, and the Wilcoxon matched pair test for comparing inside groups; p-values < 0.05 were considered to be significant.

Results

1 Clinical data

There were no significant differences in the male/female ratio or in age between the two study groups (Table 1).

Table 1 Clinical characteristics of healthy controls and asthmatic patients

Parameter	Healthy control (n = 20)	Asthmatic patient (n = 21)
Age/year	40.9 ± 10.9	48.9 ± 12.8
Sex(M/F)	17/3	17/4
Respiratory rate/breath · min^{-1}	15 - 21	25 - 46
Heart rate/beat · min^{-1}	60 - 81	110 - 168
FEV1/% of predicted	90 ± 8	60 ± 10
PEF/% of predicted	90 ± 7	60 ± 10
WBC(×10^{-9})/L	5.5 ± 1.2 (Normal)	6.4 ± 1.5 (Normal)
Neutrophil/% of total WBC number	59 ± 12	51 ± 13
Lymphocyte/% of total WBC number	41 ± 16	48 ± 19
Eosinophil/% of total WBC number	2 ± 3(0 - 6)	8 ± 8(0 - 25)

2 Comparison of IL-4 in serum and Bcl-2 expression of T cells between asthmatic patients and healthy controls

Elevated serum levels of IL-4 and up-regulated Bcl-2 expression of T cells were observed in asthmatic patients (Table 2).

Table 2 The levels of IL-4 in serum and Bcl-2 expression rate of T cells isolated from the blood of patients with asthma and healthy controls

Group	IL-4 density /pg · mL^{-1}	Bcl-2 expression rate/%
Asthmatic patient (n = 21)	280.32 ± 96.36*	29.57 ± 11.39*
Health control (n = 20)	42.13 ± 22.18	4.31 ± 3.08

IL-4 levels were detected by means of ELISA. Immunofluorescence studies were performed to detect cells with Bcl-2 markers. The comparison of difference between two groups in the Manny-Whitney u test. $\bar{x} \pm s$. *P < 0.05 *vs* health control group

3 Effect of arsenic trioxide and dexamethasone on IL-4 secretion of T cells

When T cells of both groups were cultivated *in vitro*, it can be observed that the T cells of patients with asthma released more IL-4 into the culture medium than T cells of controls. Arsenic trioxide (0.1 μg · mL^{-1}) treatment decreased the IL-4 level in the cell culture medium, but only in T cells of patients with asthma and not in the control group. However, dexamethasone (5 μg · mL^{-1}) treatment inhibited IL-4 release in both groups (Figure 1).

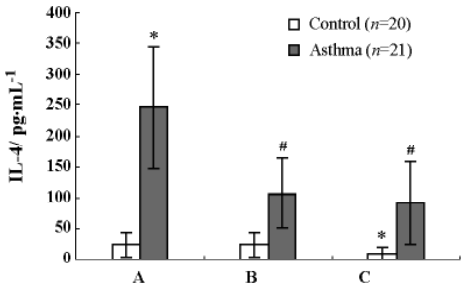

Figure 1 Effects of arsenic trioxide and dexamethasone on IL-4 release of peripheral T cells isolated from the blood of patients with asthma and healthy controls. T cells (5×10^5) were treated with 0.1 μg · mL^{-1} arsenic trioxide and 5 μg · mL^{-1} dexamethasone for 24 h. Supernatants from T cells were stored for no longer than 3 weeks. IL-4 density in supernatants was detected by ELISA. A: Not induced, spontaneous apoptosis; B: Arsenic trioxide (0.1 μg · mL^{-1})-induced apoptosis; C: Dexamethasone (5 μg · mL^{-1})-induced apoptosis. *P < 0.05 *vs* A (control); #P < 0.05 *vs* A (asthma)

4 Effect of arsenic trioxide and dexamethasone on T lymphocyte apoptosis

4.1 Results of apoptosis measurement using fluorescence microscopy A minimum of 500 cells was scored under the fluorescence microscope and categorized as normal, apoptotic, or necrotic. "Healthy" cells had a normal green nucleus, and apoptotic cells had condensed or fragmented bright green chromatin. Late apoptotic cells with secondary necrosis displayed condensed or fragmented orange chromatin (Figure 2). Both apoptotic types of cells were included in the "apoptotic category". The results were expressed as the percentage of apoptotic cells (Table 3).

When T cells of both groups were cultivated *in vitro*, the cells of asthmatic patients exhibited lower spontaneous apoptosis than the control cells. Arsenic trioxide (0.1 μg · mL^{-1}) treatment significantly increased the apoptosis rate in cells of the asthmatic group and only slightly affected the cells in the control group. When the cells were treated with dexame-

thasone (5 μg · mL^{-1}), an increase in the percentage of apoptotic cells was observed in both groups. No difference was seen in the level of dexamethasone-induced apoptosis between the control and asthmatic groups.

Table 3 The influence of arsenic trioxide and dexamethasone on apoptosis of peripheral T cells isolated from the blood of patients with asthma and healthy controls

Treatment	Control/% (n = 20)	Asthma/% (n = 21)
A	20.7 ± 4.3	13.8 ± 4.0 *
B	24.1 ± 3.3	24.0 ± 5.5 #
C	36.6 ± 10.1 *	29.0 ± 9.6 #

Apoptotic cells were counted under the fluorescent microscope after staining with acridine orange and ethidium bromide. A minimum of 500 cells was scored under the fluorescence microscope and categorized as normal, apoptotic, or necrotic. Both apoptotic and necrotic types of cells were included in the "apoptotic category". The results were expressed as the percentage of apoptotic cells. A: Not induced, spontaneous apoptosis; B: Arsenic trioxide (0.1 μg · mL^{-1})-induced apoptosis; C: Dexamethasone (5 μg · mL^{-1})-induced apoptosis. * $P < 0.05$ *vs* A (control); # $P < 0.05$ *vs* A (asthma)

4.2 Results of apoptosis measurement using flow cytometry DNA content analysis confirmed these results using fluorescence microscopy, indicating a low level of spontaneous apoptosis of T cells in patients with asthma and an induction of apoptosis by arsenic trioxide and dexamethasone treatment. While T cells of asthmatic patients showed the same sensitivity to dexamethasone treatment as those of the healthy controls, they did show more sensitivity to arsenic trioxide treatment than those from healthy subjects (Table 4, Figure 3).

Table 4 The influence of arsenic trioxide and dexamethasone on apoptosis of peripheral T cells isolated from the blood of patients with asthma and healthy controls

Treatment	Control/% (n = 20)	Asthma/% (n = 21)
A	18.9 ± 3.8	11.6 ± 3.9 *
B	23.1 ± 3.3	23.9 ± 4.8 #
C	34.7 ± 8.7 *	27.5 ± 7.8 #

Apoptosis was measured by DNA content analysis. A: Not induced, spontaneous apoptosis; B: Arsenic trioxide (0.1 μg · mL^{-1})-induced apoptosis; C: Dexamethasone (5 μg · mL^{-1})-induced apoptosis. * $P < 0.05$ *vs* A (control); # $P < 0.05$ *vs* A (asthma)

4.3 Results of apoptosis measurement using ELISA kit When apoptosis of T cells was assessed by cytochrome c release from mitochondria, it was observed that spontaneous apoptosis of T cells from the asthmatic patients was significantly lower than that of the healthy controls. Dexamethasone induced apoptosis of T cells of both groups, and arsenic trioxide induced apoptosis of T cells of the asthmatic group while the effect of arsenic trioxide on apoptosis of T cells of the control group was not significant (Figure 4).

5 Role of Bcl-2 in arsenic trioxide-induced apoptosis and dexamethasone-induced apoptosis of T cells

As shown in Table 2, up-regulated Bcl-2 expression of T cells was observed in asthmatic patients. To determine whether the modulation of Bcl-2 may be involved in T cell apoptosis, Bcl-2 expression in T cells was measured.

When T cells of both groups were cultivated *in vitro* for 24 h, it was observed that T cells of patients with asthma expressed more Bcl-2. Arsenic

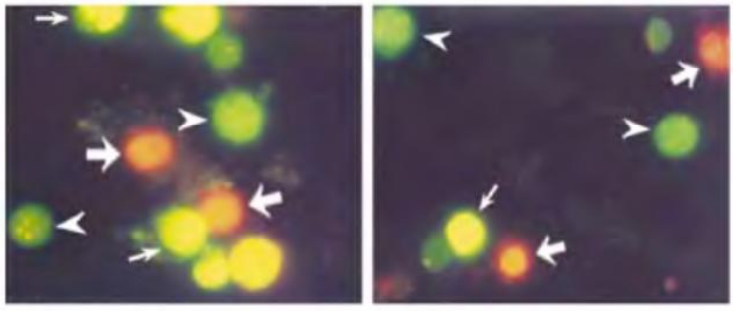

Figure 2 Representative photographs of normal and apoptotic T cells stained with acridine orange and ethidium bromide. Arrowheads indicate normal cells, fine arrows indicate apoptotic cells, and thick arrows indicate late apoptotic cells

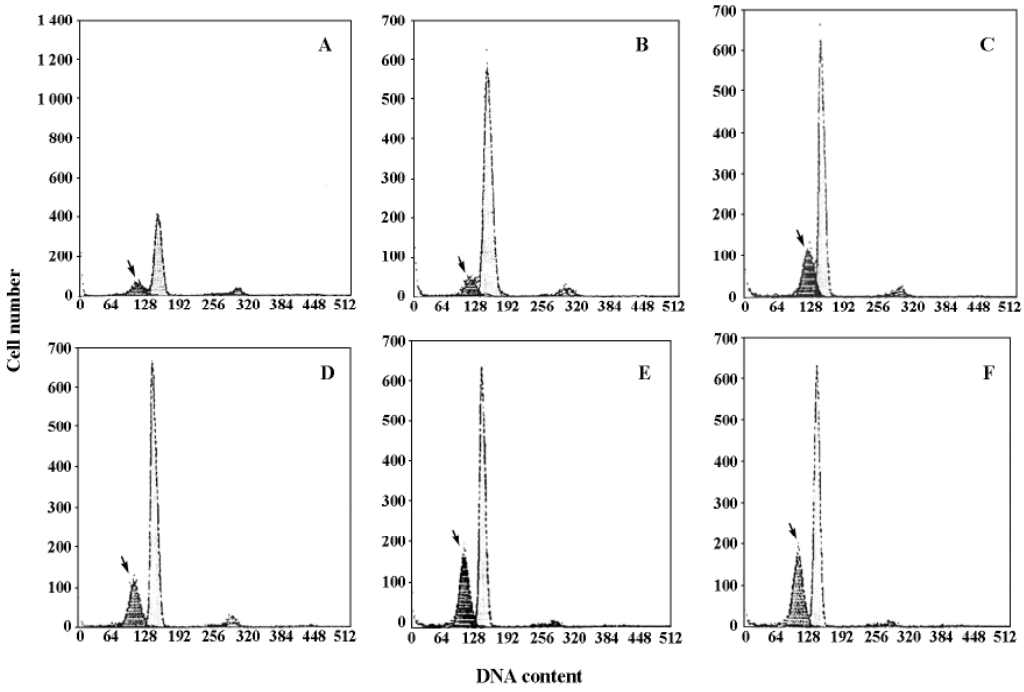

Figure 3 DNA content analysis of T cells apoptosis. Arrowheads indicate DNA subdiploid-peak. A, B, C: T cells of asthmatic patients; D, E, F: T cells of healthy controls. A, D: No treatment; B, E: Arsenic trioxide (0.1 μg · mL^{-1}) treatment; C, F: Dexamethasone (5 μg · mL^{-1}) treatment

trioxide treatment significantly down-regulated Bcl-2 expression of T cells of the asthmatic group, and slightly affected the control group. However, the effect of dexamethasone on Bcl-2 expression of T cells was not visible in both groups (Figure 5, Table 5).

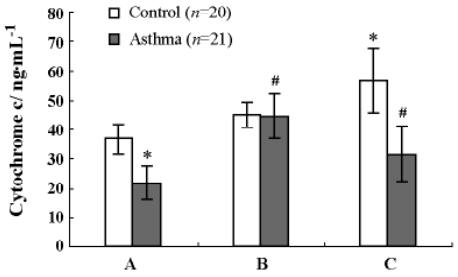

Figure 4 Influence of arsenic trioxide and dexamethasone on apoptosis of peripheral T cells isolated from the blood of patients with asthma and healthy controls. See Figure 1 for cell treatments. Apoptosis of cells was measured as the amounts of cytochrome c released from mitochondria of treated cells into the cytosol. A: Not induced, spontaneous apoptosis; B: Arsenic trioxide (0.1 μg · mL^{-1})-induced apoptosis; C: Dexamethasone (5 μg · mL^{-1})-induced apoptosis. $^{*}P < 0.05$ *vs* A (control); $^{\#}P < 0.05$ *vs* A (asthma)

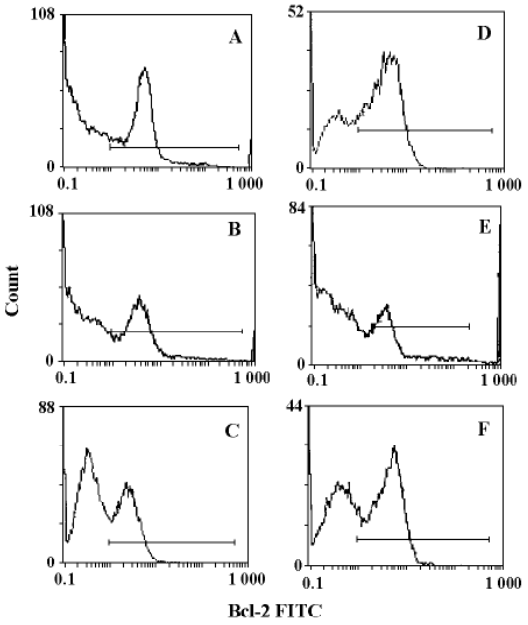

Figure 5 Flow cytometric analysis of Bcl-2 expression in T cells. A, B, C: T cells of asthmatic patients; D, E, F: T cells of healthy controls. A, D: No treatment; B, E: Arsenic trioxide (0.1 μg · mL^{-1}) treatment; C, F: Dexamethasone (5 μg · mL^{-1}) treatment

Table 5 The influence of arsenic trioxide and dexamethasone on Bcl-2 expression of peripheral T cells isolated from the blood of patients with asthma and healthy controls

Treatment	Control/% ($n=20$)	Asthma/% ($n=21$)
A	21.9 ± 5.3	36.2 ± 5.8*
B	17.8 ± 4.6	21.4 ± 7.7#
C	20.8 ± 9.5	33.6 ± 9.0

Immunofluorescence studies were performed detecting cells with Bcl-2. A: Not induced, spontaneous apoptosis; B: Arsenic trioxide (0.1 μg · mL^{-1})-induced apoptosis; C: Dexamethasone (5 μg · mL^{-1})-induced apoptosis. *$P<0.05$ *vs* A (control); #$P<0.05$ *vs* A (asthma)

Discussion

The results of our study show that T cells isolated from the blood of asthmatic patients, when compared to T cells from healthy controls, exhibit decelerated spontaneous apoptosis after a 24 h incubation *in vitro*, corresponding with other reports[10]. In investigating the causes of decelerated spontaneous apoptosis of T cells of asthmatic patients, we estimated the Bcl-2 expression and detected that T cells of asthmatic patients had a high Bcl-2 expression in comparison to controls. Since Bcl-2 is known as an anti-apoptotic gene, it seems likely that up-regulation of Bcl-2 expression of T cells is one of the causes of the decrease in apoptosis of T cells in asthmatic patients. We suppose that Bcl-2 might provide survival signals for T cells, which may result in an increase of activated T cells and IL-4 secretion, followed by persistent infiltration of allergic inflammatory cells such as eosinophils, lymphocytes and mast cells into the airways during an asthma attack.

Glucocorticoids can induce apoptosis[11,12]; therefore we chose dexamethasone as a positive control. We have confirmed that, with dexamethasone treatment, an observed increase in the percentage of apoptosis of T cells was not significantly different between the two groups irrespective of the method used. Dexamethasone treatment appeared to inhibit IL-4 release in both groups. Arsenic trioxide treatment, however, showed a significant increase in both the percentage of apoptosis of T cells and in inhibition of IL-4 release in the asthmatic group only. In other words, T cells from asthmatic patients appear to be very sensitive to both arsenic trioxide and dexamethasone treatments. In contrast, T cells from healthy persons exhibit resistance to arsenic trioxide treatment. Considering possible reasons for such resistance, we postulate that the treatment mechanism of arsenic trioxide for asthma is different from that of dexamethasone; further studies will have to be conducted to confirm this statement.

Asthma is clinically characterized by airway eosinophilic inflammation and hyperreactivity. Our current understanding of the pathophysiology of asthma is that it is an inflammatory airway disease based on an immune response, as a result of complex interactions between host and environmental factors. The onset of asthma involves a perturbation of the balance between T lymphocyte proliferation and apoptosis[13]. Emerging evidence suggests that the development of clinical sensitivity to inhaled antigens involves the establishment of a dominant population of CD4$^+$ T cells that are either classified as Th2-like or Th1-like[14]. Th2 responses are characterized by secretion of the cytokine IL-4, which is produced during allergic responses and induces the production of IgE by B cells[15,16]. In asthmatics, airway inflammation and hyperreactivity have been shown to depend upon CD4$^+$ T cells[17,18] and the cytokine IL-4[19]. Our experiments also show elevated serum levels of IL-4 in the asthmatic patients. Moreover, T cells of the asthmatic patients released more IL-4 *in vitro* than the cells from the healthy controls. IL-4 has been identified as the major cytokine involved in promoting Th2 differentiation and, as such, has become the hallmark characteristic of a type 2 response[20,21]. Therefore, we can speculate that a dominant population of T cells is Th2-like in the asthmatic patient.

T cell apoptosis is mainly mediated by two pathways: the death receptor pathway and the mitochondrial pathway. In the death receptor pathway, the Fas/FasL system plays a major controlling role. In the mitochondrial pathway, regulation is done by the Bcl-2 homologue. Glucocorticoids, such as dexamethasone, are the most effective anti-inflammatory drugs used in the treatment of asthma, in that they can induce apoptosis of T cells. Induction of T cell apoptosis by glucocorticoids was first characterized by Wyllie[22] and has been studied extensively by other investigators. Many researchers now deal with dexamethasone-induced T cell apoptosis. Some studies indicate that glucocorticoids can induce peri-blood T cell apoptosis and can significantly enhance apoptosis of T cells which had neither been activated nor had been activated by interleukin-2 in both normal and asthmatic groups[23-25]. Dexamethasone induces

apoptosis via the death receptor pathway[26,27]. To date, there is no direct evidence for the contribution of Bcl-2 homologues to dexamethasone-induced T cell apoptosis.

Arsenic trioxide, originated from traditional Chinese medicine, has been successfully applied in the treatment of acute promyelocyte leukemia (APL)[28] and it has aroused widespread interest in international tumor research academic circles[29-32]. In recent years, the apoptosis-inducing effect of arsenic trioxide on non-tumorous cells has been emphasized[33-37]. Arsenic trioxide induces apoptosis in a variety of blood cells by activation of the mitochondrial pathway for cell death[6]. In this study *in vitro*, we confirmed the following: a) T cells of asthmatic patients exhibit up-regulated Bcl-2 expression, b) arsenic trioxide treatment decreases Bcl-2 expression in T cells of asthmatic patients, and c) dexamethasone does not influence Bcl-2 expression. We can therefore speculate that arsenic trioxide induces T cell apoptosis via the mitochondrial pathway, by decreasing Bcl-2 expression in asthmatic patients. Since T cells from healthy persons exhibit little Bcl-2 expression, these T cells exhibit resistance to arsenic trioxide-induced apoptosis.

In summary, T cells of asthmatic patients exhibit decelerated apoptosis and release more IL-4, an action that is inhibited by arsenic trioxide treatment. Down-regulation of Bcl-2 expression may be an important mechanism by which arsenic trioxide induces T cell apoptosis and decreases IL-4 release in asthmatic patients. It is clear that these results suggest a novel finding, indicating arsenic trioxide is a more selective treatment in terms of available drugs for asthma.

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