

Theophylline Suppresses IL-5 and IL-13 Production, and Lymphocyte Proliferation upon Stimulation with House Dust Mite in Asthmatic Children

Mitsuaki Kimura Ikuo Okafuji Takami Yoshida

Department of Allergy and Clinical Immunology, Shizuoka Children's Hospital, Shizuoka, Japan

Key Words

Bronchial asthma · Theophylline · House dust mite · Lymphocyte proliferation · IL-5 · IL-13

Abstract

Background: Although theophylline has long been used as a medication for bronchial asthma (BA), much remains to be elucidated about its action on Th2 cells, which play a critical role in the development of BA.

Objective: This study aimed to clarify the effect of theophylline on Th2 cells in an allergen-specific manner in children with BA. **Methods:** Peripheral blood mononuclear cells (PBMCs) from 32 children with BA were stimulated with house dust mite (HDM) or a purified HDM allergen Der f 1 in the presence or absence of theophylline at a therapeutic concentration (5–20 µg/ml). The proliferation of lymphocytes and the secretion of IL-5 and IL-13 were measured to estimate the influence of theophylline. **Results:** Theophylline at a concentration of 20 µg/ml significantly suppressed lymphocyte proliferation induced by 6-day culture with HDM or Der f 1. It also significantly reduced the production of IL-5 and IL-13 upon stimulation with HDM or Der f 1. Suppression of cytokine production and lymphocyte proliferation increased with concentrations of theophylline between 5 and 20 µg/ml.

Conclusion: Theophylline suppresses the proliferation of

lymphocytes and the production of proinflammatory Th2 cytokines, IL-5 and IL-13 induced by stimulation of PBMCs with HDM in children with BA.

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Introduction

Theophylline has been used for bronchial asthma (BA) for many years [1, 2]. The antiasthmatic action of theophylline has been ascribed to the relaxation of constricted bronchial smooth muscle through an increase in the concentration of intracellular cAMP. However, the significance of this action is presently questioned because theophylline causes only a weaker bronchodilation compared with adrenergic reagents at a concentration recommended for clinical use [3]. Alternatively, theophylline has recently attracted attention because of a suppressive effect on the late asthmatic response (LAR) [4, 5]. This effect seems to be due to downregulation of allergic inflammation by theophylline [6–9].

Allergen-specific Th2 cells are thought to play a pivotal role in the development of BA by producing various cytokines causing allergic inflammation [10–12]. IL-5 is a well-known Th2 cytokine that enhances proliferation and activation of eosinophils (Eos) [13, 14], primary effector cells in allergic inflammation [15, 16]. The secretion of

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Correspondence to: Dr. Mitsuaki Kimura
Department of Allergy and Clinical Immunology
Shizuoka Children's Hospital
Urushiyama 860, Shizuoka City 420-8660 (Japan)
Tel. +81 54 247 6251, Fax +81 54 248 4763, E-Mail kimuraco@jun.ncvc.go.jp

IL-5 is demonstrated to be increased in the bronchial tissue of BA patients [17]. IL-13 is another Th2 cytokine [18] that is shown not only to enhance IgE synthesis [19], but also to directly induce inflammation in the bronchial tissue similar to that seen in patients with BA [20–23]. It also recruits Eos into the lung [24, 25]. The production of IL-5 as well as IL-13 by peripheral blood mononuclear cells (PBMCs) upon stimulation with house dust mite (HDM), a major inhalant allergen [26], is markedly elevated in patients with BA [27–29]. Lymphocyte proliferation in response to HDM, which reflects a clonal expansion of HDM-specific Th cells, is also increased in patients with BA [30, 31].

Recently, besides an inhibitory effect on the release of chemical mediators from mast cells/basophils by blocking adenosine receptors [32], various actions of theophylline on inflammatory effector cells such as Eos [33–39] or neutrophils [40, 41] were demonstrated. Moreover, theophylline is suggested to directly suppress the function of Th2 cells since the administration of theophylline lowers the levels of serum IL-4 and IL-5 [42]. Although the action of theophylline on Th2 cells was also studied in various *in vitro* systems, no consensus on the inhibition of Th2 cytokine production by theophylline has been reached yet [43–47]. In this study, we attempted to clarify a direct influence of theophylline on Th2 cells in an allergen-specific manner by the use of PBMCs from asthmatic children. The results clearly demonstrated that theophylline suppresses the production of IL-5 and IL-13 as well as lymphocyte proliferation induced by stimulation with HDM.

Materials and Methods

Subjects

Thirty-two children with BA who visited our institute between May 2000, and January 2003, were enrolled in this study after informed consent was obtained. BA was diagnosed if a patient had recurrent episodes of acute onset of dyspnea with wheezing improved by treatment with bronchodilators. The serum level of IgE antibody specific to HDM (HDM-IgE) was elevated in all subjects (table 1). No subjects were administered systemic corticosteroids, and the examination was performed during a symptom-free period.

Cell Culture

PBMCs were collected from heparinized peripheral blood by centrifugation over lymphocyte separation medium (ICN Pharmaceutical Inc., Tokyo, Japan) and resuspended at a concentration of 1×10^6 cells/ml in RPMI-1640 medium (Sigma Aldrich Fine Chemicals, Tokyo, Japan) supplemented with 10% fetal calf serum (Xavier Investments, Wacol, Australia), 2 mM glutamine and antibiotics (100 U/ml penicillin and 100 µg/ml of streptomycin). The cell suspension was distributed in duplicate on a 48-well culture plate (0.5 ml/well;

Table 1. Profile of subjects

Number	32
Age, years	7 (1–15)
Male/female	19/13
Serum IgE, IU/ml	387.4 (5.4–5,973.1)
HDM-IgE, UA/ml	85.96 (0.65–161.80)

Age, serum IgE and HDM-IgE are shown as medians and ranges (in parentheses). HDM-IgE was measured with the CAP-RAST RIA kit.

Falcon 3078, Becton Dickinson & Co., N.J., USA), and cells were cultured for 2 or 6 days in a humidified atmosphere of 5% CO₂ at 37 °C with 50 µg/ml HDM crude extract (Torii Pharmaceutical Co., Tokyo, Japan) or 2 µg/ml of purified Der f 1 protein (Seikagaku Kogyo, Co., Ltd., Tokyo, Japan) in the presence or absence of theophylline (5–20 µg/ml) (Sigma). Under these conditions, the level of endotoxin in HDM- and Der f 1-containing medium was less than 0.2 ng/ml.

After incubation, the supernatant was stored at –70 °C for measurement of cytokines, while cells were washed twice with PBS and fixed with 70% ethanol for examination of lymphocyte proliferation.

Measurement of HDM-Specific Lymphocyte Proliferation

Allergen-specific lymphocyte proliferation was measured by flow cytometry as described previously [48]. Briefly, the fixed cells were incubated in 500 µl PBS containing 100 µg/ml RNase (Sigma) at 37 °C for 1 h. Cellular DNA was stained by adding propidium iodide (Sigma) at a final concentration of 40 µg/ml. The amount of DNA per cell was measured by flow cytometry (EPICS Elite, Beckman-Coulter KK, Tokyo, Japan) and the percentage of S-phase cells that appeared in the flat region between the peaks of G₀/G₁- and G₂/M-phase cells in a DNA histogram was measured. Then, it was converted to the stimulation index using the following equation and expressed as stimulation index measured by flow cytometry (SIF).

$$\text{SIF} = (\text{S-phase cell (\%)} \text{ in stimulated sample} / \text{S-phase cell (\%)} \text{ in unstimulated sample}) \times 100.$$

Measurement of Cytokines and IgE Antibody

IL-5 and IL-13 in the supernatant were measured with ELISA kits (Immunotech), whose sensitivities were both 1 pg/ml. Although the level of IL-13 shown here was about 5-fold higher than that measured by another kit [29], there was a tight positive correlation between them ($r = 0.94$, $p < 0.001$, $n = 6$).

Serum IgE was measured using a radioimmunoassay kit (Ab beads IgE kit Eiken, Eiken Chemical Co., Ltd., Tokyo, Japan). HDM-IgE was measured using a CAP-RAST RIA kit (Pharmacia KK, Tokyo, Japan).

Statistics

All values were expressed as medians and ranges. The significance of the changes in cytokine production and lymphocyte proliferation was estimated by the Wilcoxon signed rank-sum test and regarded as significant if the *p* value was lower than 0.05.

Table 2. Suppression of lymphocyte proliferation by theophylline (n = 24)

	SIF		Suppression %
	HDM	HDM+T	
Median	204.5	164.5*	21
Range	104–721	91–479	0–42

Proliferation of lymphocytes is expressed as SIF, and the suppression of lymphocyte proliferation is shown as the percentage point. Values are shown as medians and ranges. T = Theophylline (20 µg/ml). * p < 0.0001.

Fig. 1. Dose-response curve of the suppression of lymphocyte proliferation by theophylline. The relation of the dose of theophylline with the suppression of lymphocyte proliferation is shown. The vertical axis shows the percent decrease in lymphocyte proliferation.

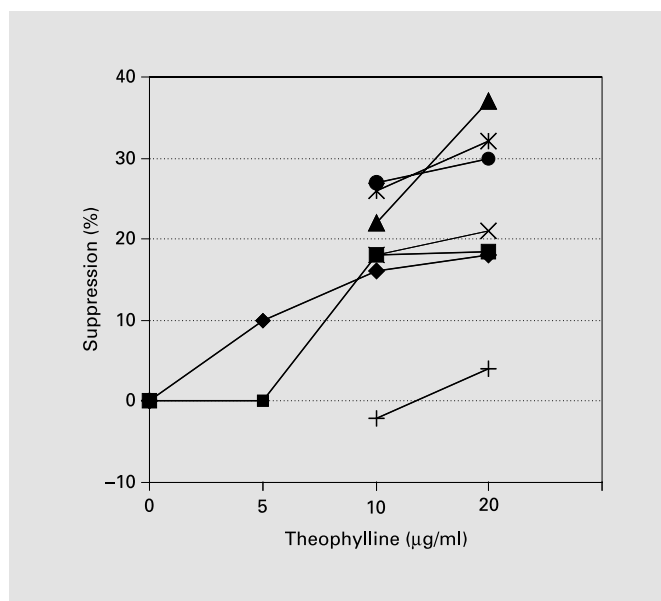


Table 3. Suppression of cytokine production

	2 days of culture			6 days of culture		
	production pg/ml		suppression, %	production pg/ml		suppression, %
	HDM	HDM+T		HDM	HDM+T	
<i>IL-5</i>						
Median	15	7.1*	48	153	113**	29
Range	5.5–62	2.6–50	6–54	49–380	35–363	4–43
n	8	8	8	9	9	9
<i>IL-13</i>						
Median	8.8	5.6**	41	215	131**	36
Range	2.4–53	1.3–22	6–58	29–772	18–376	9–48
n	10	10	10	9	9	9

The production of cytokine and its suppression are shown as medians and ranges.

T = Theophylline (20 µg/ml); n = number of subjects. * p < 0.02, ** p < 0.01.

Results

Suppression of Lymphocyte Proliferation by Theophylline

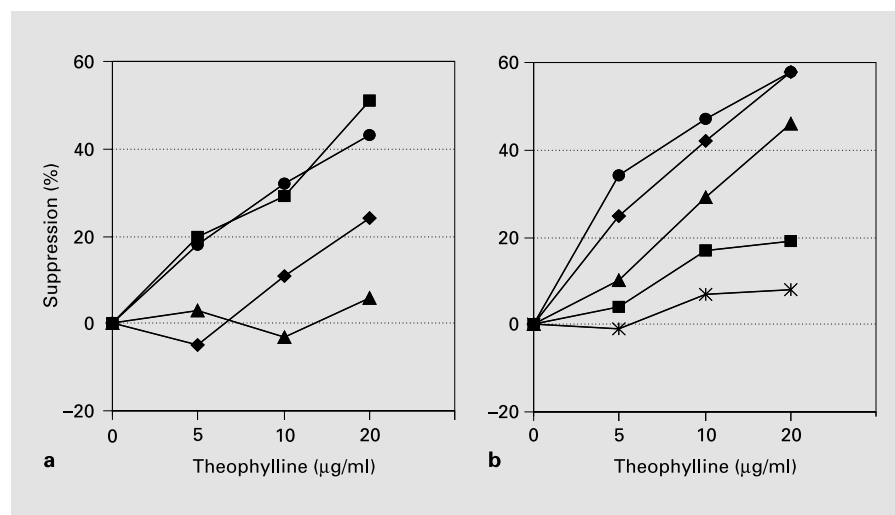
Stimulation of PBMCs from asthmatic children with HDM caused manifest lymphocyte proliferation (table 2). Twenty-five of 32 subjects (78.1%) showed an HDM-SIF level higher than the upper limit of the normal range (155) [48]. HDM-SIF decreased in a dose-dependent manner by

addition of theophylline at a therapeutic concentration (5–20 µg/ml) (fig.1). Theophylline at 20 µg/ml reduced HDM-SIF by 21% (median SIF from 204.5 to 164.5, p < 0.0001) (table 2).

Suppression of IL-5 Production by Theophylline

PBMCs from children with BA produced a large amount of IL-5 upon stimulation with HDM for 6 days (table 3). Theophylline at 20 µg/ml suppressed IL-5 pro-

Fig. 2. Dose-response curve of the suppression of cytokine production by theophylline. The relation of the dose of theophylline with the suppression of cytokine production is shown. The vertical axis indicates the percent decrease of IL-5 (a) and IL-13 production (b).



duction by HDM-stimulated PBMCs by 29% (median production from 153 to 113 pg/ml, $p < 0.01$).

IL-5 production could be detected after 2 days of incubation with HDM, when no lymphocyte proliferation was seen, in 8 of 9 patients, although the amount was smaller than that obtained after 6 days of culture (table 3). At this time point, a significant reduction in IL-5 production was also seen by addition of theophylline at 20 µg/ml (median suppression 48%) (median production from 15 to 7.1 pg/ml, $p < 0.02$) (table 3).

Suppression of IL-13 Production by Theophylline

IL-13 production could be detected in all children with BA after 2 days incubation of PBMCs with HDM (table 3). Addition of theophylline at 20 µg/ml suppressed IL-13 production by 41% (median production from 8.8 to 5.6 pg/ml, $p < 0.01$) (table 3). Theophylline also suppressed IL-13 production after 6 days of culture with HDM by 36% (median production from 215 to 131 pg/ml, $p < 0.01$).

Relation between Dose and Effect of Theophylline

As low as 5 µg/ml of theophylline was able to down-regulate IL-5 and IL-13 production (fig. 2). The suppression of cytokine production increased with the dose of theophylline up to 20 µg/ml.

Suppression of Der f 1-Induced Lymphocyte Reactions

There was a significant positive correlation between lymphocyte reactions caused by HDM crude extract and those caused by a purified HDM protein Der f 1 (proliferation, $r = 0.69$, $p < 0.05$; IL-5 production $r = 0.71$, $p < 0.05$; IL-13 production, $r = 0.80$, $p < 0.02$) ($n = 8$). Theophylline significantly suppressed the lymphocyte proliferation and the production of IL-5 and IL-13 induced by stimulation with Der f 1 (table 4).

Table 4. Effects of theophylline on Der f 1-stimulated PBMCs ($n = 8$)

	Der f 1	Der f 1 + T	Suppression, %
<i>Proliferation (SIF)</i>			
Median	201	138**	29
Range	110–406	105–281	5–44
<i>IL-5, pg/ml</i>			
Median	123	37.3*	46
Range	12.5–380	10.2–247	18–77
<i>IL-13, pg/ml</i>			
Median	97.5	29.4**	64
Range	16.0–300	6.0–120	6–82

Suppression of lymphocyte proliferation and production of cytokine induced by stimulation of PBMCs with Der f 1 for 6 days are presented. Data are expressed as medians and ranges. T = theophylline (20 µg/ml). * $p < 0.05$, ** $p < 0.02$.

Discussion

The production of proinflammatory Th2 cytokines, IL-5 and IL-13, by PBMCs stimulated with a major inhalant allergen, HDM, is markedly high in children with BA [28,

29]. In this study, we first demonstrated in an allergen-specific manner that theophylline decreases the production of these cytokines by HDM-stimulated PBMCs from asthmatic children. Considering the significance of IL-5 and IL-13 in the pathogenesis of BA, this seems to be an important mechanism by which theophylline mitigates allergic inflammation in the bronchial tissue and helps to control symptoms of asthmatic children.

Recently, Yoshida et al. [47] reported that theophylline did not reduce the production of IL-13 by PBMCs from atopic asthmatics at a therapeutic concentration. Although the actual cause of the discrepancy in the results is a subject of further study, it may be related to the difference in experimental methods. They induced cytokine production by stimulating PBMCs with phytohemagglutinin, a potent polyclonal mitogen, while we stimulated PBMCs with HDM, a major inhalant allergen. Although the production of IL-13 was about 10-fold lower, the present system mimics more precisely the *in vivo* mode of activation of T cells. In other words, the present system contains the process of antigen presentation by monocytes/macrophages and the signal transduction through T cell receptor.

Lymphocyte proliferation in response to HDM is increased in patients with BA [30, 31]. Proliferating cells upon stimulation with HDM were shown to have Th phenotypes [31]. Theophylline was reported to down-regulate lymphocyte proliferation induced by polyclonal mitogens [43, 44]. We here examined whether theophylline at a therapeutic concentration suppresses the proliferation of lymphocytes stimulated with HDM. As a result, it was confirmed that theophylline significantly suppresses lymphocyte proliferation upon stimulation with HDM in a dose-dependent manner. Suppression of lymphocyte proliferation may also contribute to the reduction of Th2 cytokine production by preventing clonal expansion of HDM-specific Th cells.

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