

Theophylline inhibits the differentiation of human monocyte into dendritic cell potentially via adenosine receptor antagonism

K. Yasui, Y. Kondo, T. Wada, M. Yashiro, M. Tsuge and T. Morishima

Department of Pediatrics, Dentistry and Pharmaceutical Sciences, Okayama University Graduate School of Medicine, Okayama, Japan

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Summary

Background Theophylline has an anti-inflammatory action that may account for its clinical effectiveness in the reduction of inflammatory cells in the airways. Dendritic cells (DCs) are professional antigen-presenting cells, capable of priming naïve T cells, and play key roles in the activation of immune responses in asthma.

Objective The purpose of this study was to investigate the effects of theophylline on human monocyte differentiation into DCs and whether this involved antagonism of adenosine receptors.

Methods Peripheral human blood monocytes were cultured in the presence of granulocyte/macrophage-colony stimulating factor and IL-4 to induce DC differentiation. The cells were incubated with theophylline, KF17837 (a selective A2a receptor antagonist) and enprofylline (A2b receptor antagonist) and co-incubated with selective adenosine A1 and A2a receptor agonists, a phosphodiesterase inhibitor (rolipram) and adenosine deaminase (ADA) to determine their effects on DC differentiation. In addition, depletion of adenosine receptors by small interfering RNA (siRNA) was also examined.

Results Monocytes differentiated into myeloid DCs in the culture system. The number of DCs was remarkably reduced by 60–70% when theophylline was administered at a therapeutic concentration. This effect was concentration-dependently exacerbated, was partly mediated by cellular apoptosis and was effectively reversed by the addition of the A1 agonists [2-chloro- N^6 -cyclopentyladenosine, N^6 -cyclohexyladenosine, and N -ethylcarboxamidoadenosine (NECA)] or the A2a agonist (CGS-21680, NECA). The depletion of the adenosine A1 receptor by siRNA and addition of ADA remarkably reduced DC differentiation. Meanwhile, both enprofylline and rolipram had little effect. **Conclusion** Our findings suggest that the adenosine A1 (and possibly coordinated with A2a) receptors contribute to DC differentiation and survival. These findings provide further evidence that theophylline has an anti-inflammatory action in bronchial asthma.

Keywords adenosine receptor, agonist, airway inflammation, antagonist, GM-CSF, IL-4, phosphodiesterase inhibitor

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Correspondence:

Kozo Yasui, Department of Pediatrics, Dentistry and Pharmaceutical Sciences, Okayama University Graduate School of Medicine, 2-5-1 Shikata-cho, Okayama 700-8558, Japan.

E-mail: k-yasui@md.okayama-u.ac.jp

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Introduction

Bronchial asthma is a chronic inflammatory disease of the airways associated with a predominant T-helper type 2 (Th2) response to inhaled allergens leading to airway infiltration by eosinophils, mast cells and goblet cells, fibrocyte hyperplasia and bronchial hyperreactivity [1]. Effector Th2 lymphocytes are intimately involved in controlling the various aspects of the disease. Meanwhile, dendritic cells (DCs) derive from peripheral monocytes/

macrophages and are the most important antigen-presenting cells (APCs) in the immune system *in vivo* [2–4]. Airway DCs form a network in the bronchial epithelium, capture inhaled antigens and can migrate to the mediastinal lymph nodes, where the antigens are presented to recirculating naïve CD4⁺ and CD8⁺ T cells [5–10]. CD11c⁺ DC are key pro-inflammatory cells that are necessary and sufficient for Th2 cell stimulation during ongoing airway inflammation and activate eosinophilic inflammation [9].

Theophylline, a methylxanthine derivative and a weak non-specific phosphodiesterase inhibitor and once widely used for asthma symptom control, has fallen into disfavour in recent years because of concerns about side-effects and the inconvenience of monitoring blood levels [11]. Previous studies, however, suggest that theophylline has significant anti-inflammatory and immunomodulatory effects at lower concentrations (< 10 mg/L), and can be given as once-daily oral medication, which does not require monitoring of blood levels [12]. The inhibition of late asthmatic responses by theophylline has been shown to involve the regulation of the influx and inhibition of neutrophil activation [13, 14]. These effects of theophylline occur at serum concentrations *in vitro* within the accepted therapeutic range [14]. Low-dose theophylline and montelukast have similar adherence, efficacy and tolerability in the study; in addition, theophylline is considerably less expensive and safe for patients not using inhaled corticosteroids (ICS) [15]. For patients who cannot use or do not need ICS, low-dose theophylline is recommended as an inexpensive alternative asthma controller therapy and should be applied more worldwide [15].

Meanwhile, the precise anti-inflammatory mechanism of theophylline is not fully understood. Especially, there have been few studies investigating the pharmacological actions of theophylline on the activities of monocytes/DCs. Among the studies that have been reported, Kidney *et al.* [16] described a significant decline in peripheral blood monocytes in patients with a plasma theophylline level > 5 mg/L, and this decline was associated with decreased number of activated CD4 T lymphocytes ($CD4^+/CD25^+$) and CD8 T cells ($CD8^+/HLA-DR^+$). It has been well established that peripheral blood monocytes cultured *in vitro* in the presence of the appropriate cytokines, such as granulocyte/macrophage-colony stimulating factor (GM-CSF) and IL-4, differentiate into strongly CD11c-positive DCs [17, 18]. The present study was undertaken to evaluate the effects of theophylline on the differentiation of human monocytes to dendritic cells and to determine the pharmacological mechanism that underlies these effects in order to clarify the anti-inflammatory potential and the appropriate use of theophylline for the management of bronchial asthma. Recent studies suggested that the maturation and immune functions of murine DCs are modulated via antagonism of adenosine A2b receptors [19, 20]; however, this is the first report studying DC proliferation in human cells.

Materials and methods

Reagents

RPMI-1640 and phosphate-buffered saline (PBS) were obtained from GIBCO (Grand Island, NY, USA). Theophylline monohydrate (water soluble), enprofylline, adenosine

deaminase (ADA; type V), N^6 -cyclohexyladenosine (CHA), 2-chloro- N^6 -cyclopentyladenosine (CCPA), 5'- N -ethylcarboxamidoadenosine (NECA), dimethyl sulphoxide (DMSO), Histopaque 1077 and HEPES were purchased from Sigma (St. Louis, MO, USA) and Wako Pure Chemical Industries (Osaka, Japan). 2- p -(2-carboxyethyl) phenethylamino-5'- N -ethylcarboxamidoadenosine (CGS-21680) and a specific phosphodiesterase χ inhibitor (rolipram) were from Funakoshi Co. (Tokyo, Japan). (*E*)-1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine (KF-17837, a selective adenosine A2a receptor antagonist, [21]) was provided by Kyowa Hakko (Tokyo, Japan). The compounds (except for theophylline) were dissolved in DMSO, which is known as an agent that induces differentiation and morphological changes in monocytic leukaemia cell lines [22]; however, the final concentration of DMSO is $< 0.1\%$ in our experiments. It had little effect on the pH of culture media and little effect on monocyte-macrophage differentiation as well as reported previously [23]. Theophylline monohydrate was then diluted in PBS. Recombinant human cytokines (GM-CSF and IL-4) were obtained from R&D systems (Abingdon, UK). Fluorochrome-conjugated monoclonal antibodies were from Becton Dickinson (San Jose, CA, USA) for flow cytometry analysis. Anti-mAb-coated magnetic beads and the magnetic cell sorter system were purchased from Miltenyi Biotec GmbH (Bergisch Gladbach, Germany).

Monocyte separations and dendritic cell cultures

Heparinized venous blood was obtained from healthy volunteers (male, 25–45 years old) recruited from Okayama University Hospital staff. Peripheral blood mononuclear cells (PBMCs) were isolated by endotoxin-free histopaque density gradient centrifugation. Monocytes were isolated by negative selection with mAbs (CD3, CD7, CD19, CD45RA, CD56 and anti-IgE)-coated immunologic magnetic beads (Miltenyi Biotec GmbH) and a cell sorter from centrifugated PBMCs, depleting T, B-lymphocytes, NK cells, basophils and matured DCs. The purity of the isolated human monocytes, as assessed by the detection of reactive oxygen species with a fluorescence probe (hydroxyphenyl fluorescein) [24] stimulated with 10 ng/mL PMA, was $> 95\%$. Monocytes were cultured at 37°C under a 5% CO_2 atmosphere in a conditioned medium consisting of RPMI1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 50 U/mL penicillin, 50 $\mu\text{g/mL}$ streptomycin and 10 mM HEPES at 2×10^6 cells/2 mL/well in 12-well tissue culture plates (Sumitomo Bakelite, Tokyo, Japan) with 10 ng/mL GM-CSF and 10 ng/mL IL-4. At day 7 of culture, theophylline and other compounds (adenosine receptor agonists, ADA, enprofylline and rolipram) were mixed in usual experiments. All culturing procedures were performed under sterile conditions, and the cells were maintained for 5 days, at which time the medium was changed to the

same volume of medium supplemented with the same cytokines and chemical compounds. The cultured monocytes without cytokines (GM-CSF and/or IL-4) could not survive for >5 days. DCs were constantly generated by culture of peripheral blood monocytes for 7–10 days in the presence of GM-CSF and IL-4. The study protocol was in accordance with the guidelines of the Institutional Review Board of Okayama University Graduate School of Medicine, and written informed consent was obtained from the volunteers.

RNA interference

For experiments involving RNA interference, siRNA specific for adenosine receptor was transfected 48 h before the addition of GM-CSF and IL-4 according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). The following are the selected target mRNA sequences for adenosine receptors: siRNA A1: 5'-CUGGCAGCGG AAAUGGUCATT-3'; siRNA A2a: 5'-CAGGAGTGTCTGATGATTCA-3'; siRNA A2b: 5'-CACGTATCTAGCTAATATGTA-3'; siRNA A3: 5'-CCCTATCGTCTATGCCTATAA-3'; siRNA control: ACUGGUAAGGCGACGGUCTT-3'.

Cell staining and evaluation of dendritic cell formation

Culture plates were rinsed twice with PBS and dried well at day 14, and the cells were stained with May-Grünwald-Giemsa stain (Merck Ltd., Darmstadt, Germany). Digital pictures were taken at low power ($\times 20$ objective) with a microscope (Keyence, Osaka, Japan) under LV light for the quantification of the number of DCs and macrophages. The average number of total visible nuclei was calculated in four representative low-power ($\times 20$) microscopic fields (~ 300 nuclei) by an experimenter who was not aware of the experimental protocol, and the number of cells showing a typical DC morphology (see an example in Fig. 1a) was evaluated.

Flow cytometry analysis

The adhered cells were detached using 0.02% ethylenediaminetetraacetic acid (EDTA) PBS (Sigma) and stained with fluorescein-isothiocyanate (FITC)- or PE-conjugated mAbs and compounds from Becton Dickinson Biosciences (San Diego, CA, USA). The following were used: FITC-conjugated anti-HLA-DR, annexin V and PE-conjugated anti-CD11c and 7AAD. The cellular composition was determined using a FACScalibur flow cytometer.

Fluorometric assay for caspase-3-like activity

DEVD-7-amino-4-methylcoumarin cleavage was measured using a modification of the fluorometric assay of Parvathenani et al. [25]. Cultured cell lysates were centrifuged (15 000 g, 3 min) and the protein concentrations

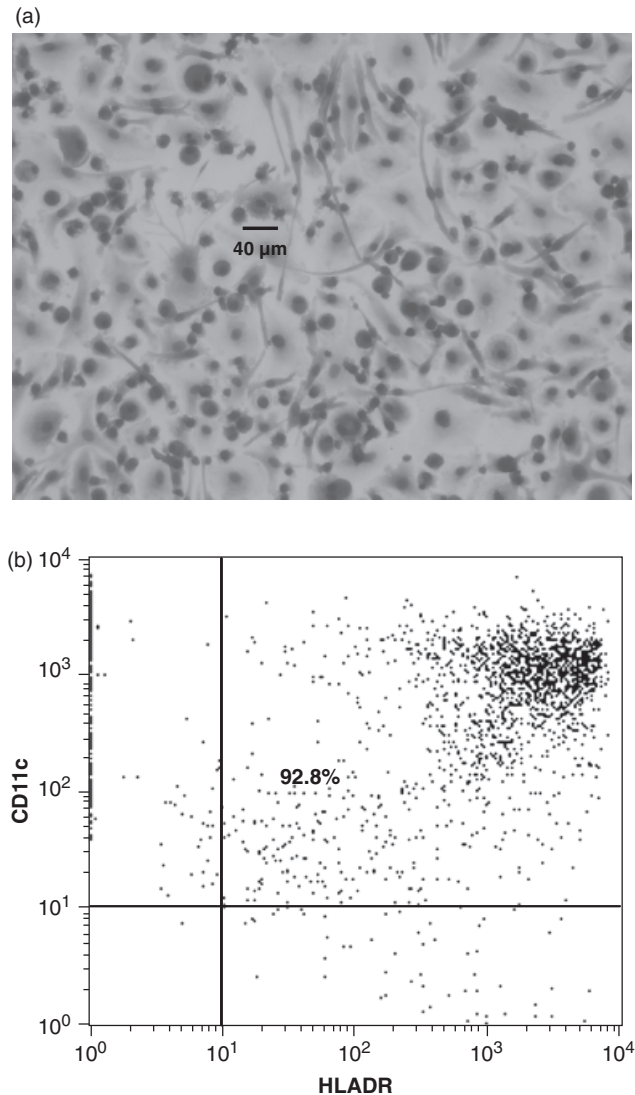


Fig. 1. Characterization of peripheral blood monocytes cultured in granulocyte/macrophage-colony stimulating factor (GM-CSF) (10 ng/mL) + IL-4 (10 ng/mL). (a) A representative image of May-Grünwald-Giemsa-stained monocyte-derived cells cultured in GM-CSF + IL-4 ($\times 20$) at day 14. Differentiated dendritic cells were detached and recognized as FITC-MHC II (HLA-DR) positive and PE-CD11c positive by FACS (b).

were determined by colorimetric analysis using protein assay reagents (BioDynamics Laboratory Inc., Tokyo, Japan). The cell lysate and the substrate DEVD-AMC (50 μ M) were combined in a standard reaction buffer (10% sucrose, 0.1% CHAPS, 10 mM DTT and 50 mM HEPES; pH 7.5). Then, the enzymatic activity was determined spectrophotometrically (BioRad, Hercules, CA, USA) for 60 min, using excitation and emission wavelengths of 355 and 460 nm, respectively.

Statistical analysis

The quantitative data are shown as the means $\pm$ SD, and were analysed using statistical software for Excel (Atoms,

Tokyo, Japan). Each experiment was performed at least in triplicate in three independent experiments. Two-tailed Student's *t*-test assuming unequal variance for all pairs was used in comparisons of groups. The results with *P*-values < 0.05 were considered statistically significant.

Results

Effect of theophylline on monocyte and dendritic cell differentiation

Exposure of peripheral blood monocytes to GM-CSF and IL-4 for 14 days led to a morphologic change to fibrous dendritic cells and a few foamy cells (Fig. 1a). These cells, detached with 0.02% EDTA solution, were not morphologically identical to DCs but they were confirmed to be positive for HLA-DR and CD11c (Fig. 1b) at day 7 of culture, which are molecules essential for antigen presentation by DCs. Peripheral blood monocytes were cocultured with or without cytokines (GM-CSF and IL-4). At days 3–5 of culture, lived monocytes were decreased in number without cytokines (data not shown). At day 7 of culture, the addition of theophylline resulted in a significant and concentration-dependent inhibition of the differentiation of human peripheral monocytes to DCs with cytokines as shown in Figs 2a and b at day 14 of culture. The number of DCs in culture was remarkably reduced by 60–70% when theophylline was administered at a therapeutic concentration (Fig. 2b). The cellular toxicity of theophylline was avoided because it is harmless to lymphocyte culture even at the highest concentration (5×10^{-4} M, 90 µg/mL) by the trypan blue dye test.

Effects of modulation of the adenosine receptor on dendritic cell differentiation

The presence of theophylline (1×10^{-4} M) reduced DC differentiation to 24 ± 12 cells per low-power ($\times 20$) microscopic field at day 14 of culture. Because four adenosine receptor subtypes have been cloned, designated A1, A2a, A2b and A3, studies were undertaken to identify the receptor involved. The co-incubation with theophylline and receptor agonists, both CHA (A1; $K_a = 2.5\text{--}75$ nM $\gg$ A2a = A2b; $K_a = 2000$ nM) and CGS-21680 (A2a; $K_a = 5\text{--}75$ nM $>$ A1; $K_a = 500\text{--}1500$ nM), resulted in a significant recovery of the number of DC-differentiated cells (Fig. 3a). The A1-specific siRNA significantly decreased the number

of DCs cultured from isolated monocytes (Fig. 3b) as well as ADA (0.1 U/mL). NECA (nonselective; A1, A2a, A2b) and CCPA (A1 $>$ A2) also counteracted theophylline. Meanwhile, KF-17837 (an adenosine A_{2A} receptor antagonist; $K_i = 9$ nM for CGS21680) [21] significantly reduced DC differentiation in a manner similar to theophylline (Fig. 3c).

Effects of a xanthine derivative and a phosphodiesterase inhibitor on dendritic cell differentiation

Theophylline is a xanthine derivative that shows an affinity (in the micromolar range) for adenosine receptors as an antagonist, does not discriminate between the A1 and A2a receptor subtypes ($K_i = 14, 18$ µM, respectively) and is a minimum potent inhibitor of phosphodiesterase (PDE) isozymes. Enprofylline has been shown to be effective as an antagonist of A2b ($K_i = 7$ µM) but not A2a ($K_i > 1000$ µM) receptors [26]. Both enprofylline and a specific PDE IV inhibitor (Rolipram, $K_i = 60\text{--}80$ nM) did not compete with or exacerbate the effects of theophylline on DC differentiation from peripheral blood monocytes (Fig. 4). This was also indicative of the effect through neither A2b receptors nor PDE inhibitor.

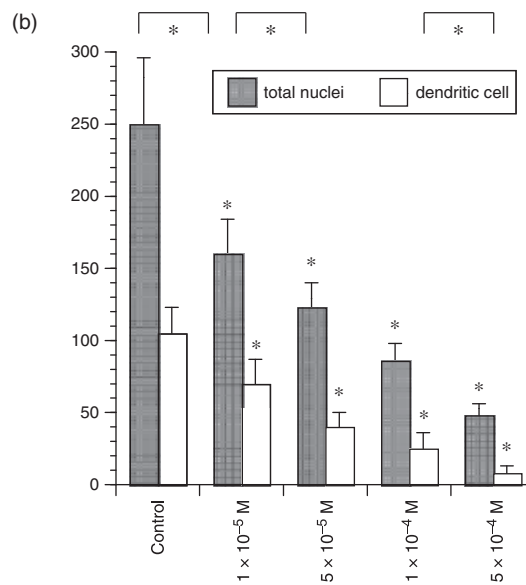
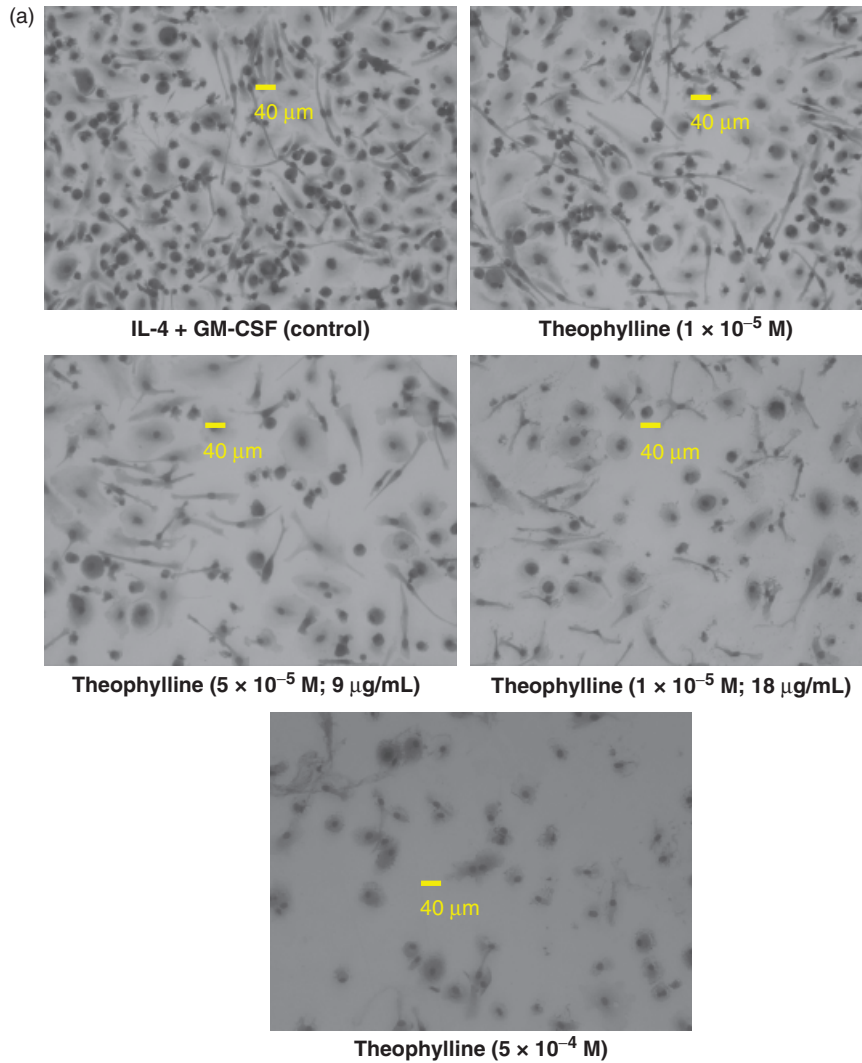
Apoptosis of dendritic cells and activated caspase-3-like activity in cell lysate

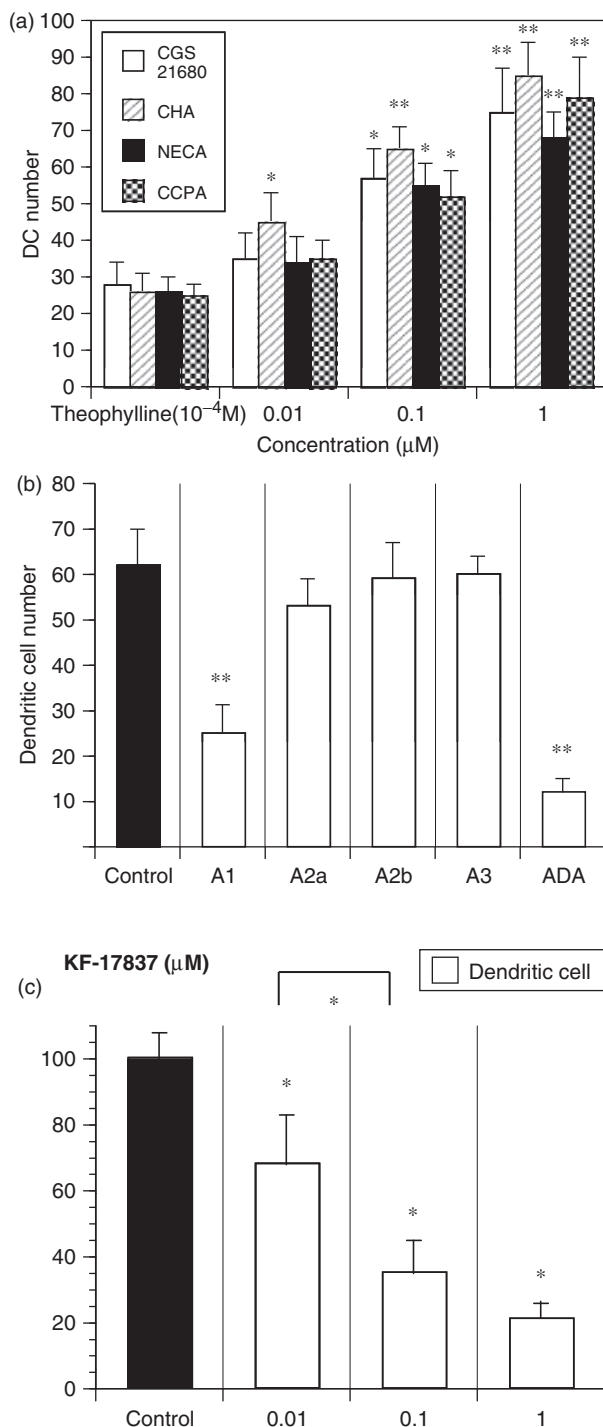
At the light microscopic level, the theophylline-treated DCs derived from monocytes demonstrated the characteristic morphology of apoptosis: segmented darkly stained pyknotic nuclei but a structurally intact cytoplasm (Fig. 5a). The cellular apoptosis induced by theophylline was confirmed by immunofluorescence staining with FITC-labelled annexin V (Fig. 5a) and flow cytometry analysis (Fig. 5b). The post-apoptotic and necrotic cells (dead cells) were considered to be positive with both annexin V and 7AAD stains (double positive). Caspase-3-like activity was measured by cleavage of the fluorogenic substrate DEVD-AMC as described previously [25]. The addition of theophylline or ADA (0.1 U/mL) significantly enhanced the caspase-3-like activity ($n = 4$, $P < 0.01$) (Fig. 5c).

Discussion

This is the first report indicating that theophylline (a xanthine derivative) interferes with DC differentiation

Fig. 2. Monocyte-derived dendritic cells (DCs) cocultured with theophylline at various concentrations. The cells were cultured for 7 days in the presence of 10 ng/mL granulocyte/macrophage-colony stimulating factor (GM-CSF) and 10 ng/mL IL-4. At day 7 of culture, the addition of theophylline resulted in a significant and concentration-dependent inhibition of the differentiation of human peripheral monocytes (day 14). Cultured cells were stained and photographed with LV light (magnification: $\times 20$). The average cell numbers in a microscopic field were analysed. Representative images of May-Grünwald-Giemsa-stained cells are shown (a). DC formation was significantly and dose-dependently affected by the presence of theophylline in the culture medium (b). Results are expressed as the means $\pm$ SD ($*P < 0.05$ between two groups, and compared with control values). The results shown are representative of three independent experiments.





from human peripheral blood monocytes. Persistent inflammation in the airways is thought to be an essential feature of chronic asthma. An inflammatory cell infiltrate exists in the airway walls even in mild asthma. CD11c^+ DCs accumulate within sites of eosinophilic airway inflammation and cluster with CD4^+ T cells in the airway submucosa and the perivascular space at areas of intense eosinophilic inflammation [6, 9]. A DC depletion study [9]

Fig. 3. (a) Effects of selective adenosine A1 receptor agonists (CHA, CCPA), a selective adenosine A2a receptor agonist (CGS-21680) and non-selective A1 and A2a agonist (NECA) on dendritic cells (DCs) cultured in the presence of GM-CSF and IL-4 for 14 days in the co-existence of theophylline (1×10^{-4} M) for 7 days. The y-axis represents the total number of DC cells with a typical morphology. The x-axis represents the molar mass and the compounds. The results shown are representative of three independent experiments. Errors bars show means $\pm$ SD ($n = 3$, ** $P < 0.01$, * $P < 0.05$ compared between two groups or compared with the value of theophylline). (b) Anti-proliferative effects of small interfering RNA (siRNA) transfection in human blood monocytes. The results shown are representative of three independent experiments. Errors bars show mean $\pm$ SD ($n = 3$, ** $P < 0.01$ compared with the value of control siRNA). ADA represents the presence of adenosine deaminase (0.1 IU/mL). (c) Effects of a selective adenosine A_{2A} receptor antagonist (KF-17837) on DCs cultured in the presence of GM-CSF and IL-4 for 7 days. The y-axis represents the total number of DC cells with a typical morphology. The x-axis represents the molar mass and the compounds. Results are expressed as the means $\pm$ SD (* $P < 0.05$ compared with control or between two groups) from four separate experiments. ADA, adenosine deaminase; CCPA, 2-chloro- N^6 -cyclopentyladenosine; CHA, N^6 -cyclohexyladenosine; NECA, N -ethylcarboxamidoadenosine; GM-CSF, granulocyte/macrophage-colony stimulating factor.

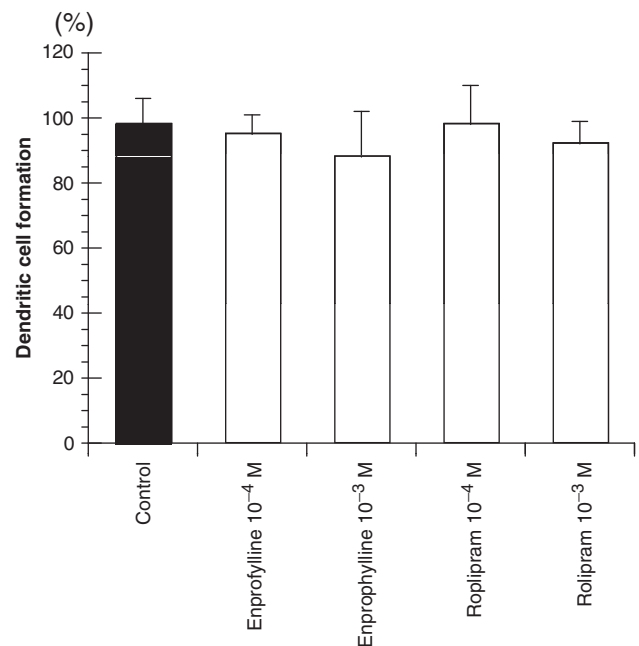


Fig. 4. Effects of enprofylline (A2b antagonist) and a specific phosphodiesterase IV inhibitor (Rolipram) on dendritic cells cultured in the presence of granulocyte/macrophage-colony stimulating factor and IL-4 for 7 days (day 14). The x-axis represents the molar mass and the compounds. The results shown are representative of four independent experiments. Errors bars show mean $\pm$ SD from four separate experiments.

has shown that CD11c^+ DCs are required for eosinophilic airway inflammation *in vivo*, and that repeated injection of pulsed DCs induces all the features of asthma in an animal model, including Th2 cytokine activation and bronchial hyperreactivity. DCs are professional APCs,

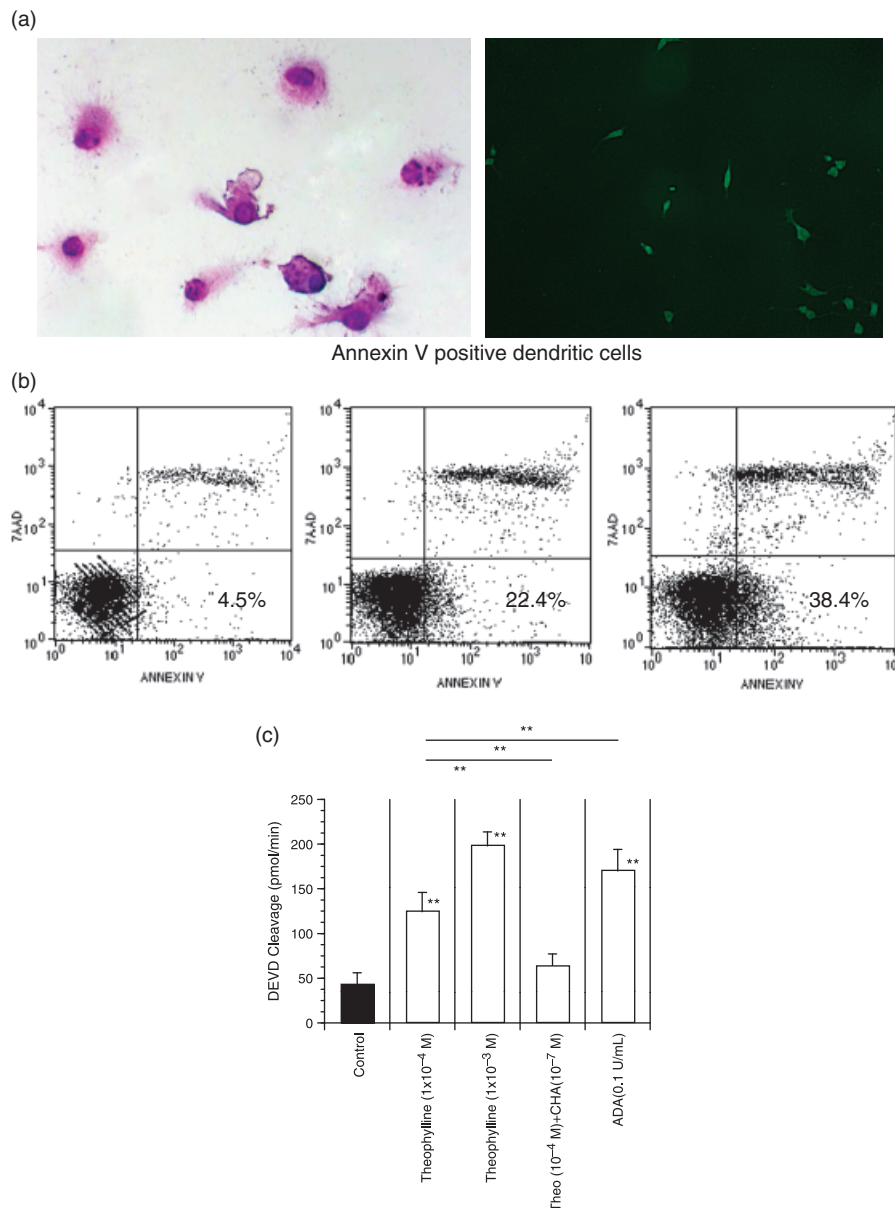


Fig. 5. (a) Morphologic features of dendritic cells (DCs) incubated with granulocyte/macrophage-colony stimulating factor and IL-4 in the presence of 5×10^{-5} M theophylline cultured for 7 days (day 14). May-Grünwald-Giemsa-stained preparation was photographed at $\times 400$. FITC-Annexin V-labelled DCs were observed at $\times 20$. (b) Flow cytometric analysis of annexin V and 7-AAD binding in DCs incubated with 5×10^{-5} M theophylline. The percentages of annexin V single-positive cells are shown. (c) Caspase-3-like activity (pmol/min/ 5×10^6 cells) in the cell lysate of cultured DCs with the compounds. Results are expressed as the means $\pm$ SD (** $P < 0.01$ between two groups) from four separate experiments.

capable of priming naïve T cells, and play the key roles in orchestrating the activated $CD4^+$ T cell-mediated allergic inflammatory reaction [8–10].

The findings of the present study show that, *in vitro*, theophylline significantly inhibited IL-4 and GM-CSF-induced DC differentiation from peripheral blood monocytes in a dose-dependent manner by mediating cellular apoptosis in part. These effects were still significant at a lower theophylline concentration (1×10^{-5} M; $1.8 \mu\text{g/mL}$). These findings are clinically relevant with the previous

finding that theophylline reduced peripheral blood monocytes in asthmatic patients on continuous treatment [16], which was associated with a decline in activated T cells. Jaffar et al. [27] also reported that low-dose oral theophylline treatment reduces the number of T lymphocytes in the airways of asthmatics. The mechanism by which this action is achieved has remained unclear so far, but may be a consequence of theophylline-induced inhibition of DC differentiation and the effects on T cell activation in the asthmatic airways. Our data could support the

previous findings by Toyoshima *et al.* [28] that long-term administration of theophylline reduced the serum IgE level in asthmatic children, and by Louis *et al.* [29] that theophylline therapy consequently reduced the number of eosinophils on sputum. It is hypothesized that the acceleration of DC apoptosis by theophylline seems to be associated with modification of T cell activation (antigen presentation) and subsequently sedation of eosinophilic airway inflammation.

In the present study, both the A1 receptor-selective agonists CCPA, CHA and the A2a receptor-selective agonist CGS21680 potently reduced the ability of theophylline to inhibit monocyte differentiation into DCs. NECA works as an agonist of adenosine A1 and A2a. On the other hand, enprofylline, which has been shown to be effective as an antagonist of A2b but not A2a receptors [26], did not affect DC differentiation of monocytes. The addition of ADA was as effective as theophyllin in interfering with DC differentiation. Rolipram (a specific phosphodiesterase inhibitor and cAMP-increasing agent) also had little effect on DC differentiation and cell viability. The therapeutic concentrations (5–20 µg/mL) of theophylline can produce only a slight inhibition of PDE activity [30, 31]. These data suggest that theophylline mainly acts as an adenosine receptor antagonist in the inhibition of DC differentiation. Activation of adenosine A1 receptors mediates cellular protective effects and induces cell proliferation and prevents apoptosis in human cells [32–34]. Although the depletion of adenosine A1 receptor but A2a, A2b, A3 by siRNA significantly reduced DC differentiation, we cannot conclude that theophylline acts as an A1 antagonist and exclude the possible involvement of the A2a receptor. The selectivity of the receptor agonists or antagonists is not definitive, and the A2a receptor-selective antagonist KF-17837 was as effective as theophyllin in interfering with DC differentiation from peripheral blood monocytes. The A1 receptors are weakly expressed on peripheral blood monocytes and are consequently expressed on cultured monocytes [35, 36]. Hence, it is possible that A1 receptors are more sensitive to siRNA interference and/or that A1 receptors can coordinate with the adenosine A2a receptors. Evidence has indicated that adenosine can alter the course of monocyte proliferation and differentiation [37]; however, the specific intracellular mechanisms through which adenosine receptor signaling influences the maturation of monocyte are unclear and remain an important area for future study.

There is increasing evidence that theophylline has anti-inflammatory effects in the airway. The anti-inflammatory effects of theophylline are seen at concentrations, which is the dose at which significant clinically useful bronchodilation is evident. Recently, it has been demonstrated that low-dose theophylline improves asthma symptom control more than a leukotriene antagonist, montelukast, and is a safe and less expensive alternative

asthma treatment for the patients not using ICS [15]. Although theophylline is currently less preferred than leukotriene antagonist or a long-acting β_2 -agonist, long-term administration of theophylline [38] (round-the-clock therapy; RTC) should be reconsidered for the management of bronchial asthma due to its anti-inflammatory effects on DC proliferation and survival for an add-on therapy with ICS.

In conclusion, here, we demonstrated that theophylline is a potential immunosuppressive agent that inhibits the differentiation of monocytes into DCs. Low-dose oral theophylline treatment may reduce the number of T lymphocytes and eosinophils in accordance with decreased numbers of DCs in the airways of asthmatics. These findings may contribute to the further understanding of theophylline's therapeutic efficacy in asthma.

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References

- 1 Wills-Karp M. Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu Rev Immunol* 1999; 17:255–81.
- 2 Herrick CA, Bottomly K. To respond or not to respond: T cells in allergic asthma. *Nat Rev Immunol* 2003; 3:405–12.
- 3 Holt PG, Schon-Hegrad MA, Oliver J. MHC class II antigen-bearing dendritic cells in pulmonary tissues of the rat. Regulation of antigen presentation activity by endogenous macrophage populations. *J Exp Med* 1988; 167:262–74.
- 4 Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature* 1998; 392:245–52.
- 5 Lambrecht BN, De Veerman M, Coyle AJ, Gutierrez-Ramos JC, Thielemans K, Pauwels RA. Myeloid dendritic cells induce Th2 responses to inhaled antigen, leading to eosinophilic airway inflammation. *J Clin Invest* 2000; 106:551–9.
- 6 Lambrecht BN, Pauwels RA, Fazekas De St Groth B. Induction of rapid T cell activation, division, and recirculation by intratracheal injection of dendritic cells in a TCR transgenic model. *J Immunol* 2000; 164:2937–46.
- 7 Jahnsen FL, Moloney ED, Hogan T, Upham JW, Burke CM, Holt PG. Rapid dendritic cell recruitment to the bronchial mucosa of patients with atopic asthma in response to local allergen challenge. *Thorax* 2001; 56:823–6.
- 8 Vermaelen KY, Carro-Muino I, Lambrecht BN, Pauwels RA. Specific migratory dendritic cells rapidly transport antigen from the airways to the thoracic lymph nodes. *J Exp Med* 2001; 193:51–60.
- 9 van Rijt LS, Jung S, KleinJan A *et al.* In vivo depletion of lung CD11c⁺ dendritic cells during allergen challenge abrogates the characteristic features of asthma. *J Exp Med* 2005; 201:981–91.

- 10 Lommatzsch M, Bratke K, Bier A *et al.* Airway dendritic cell phenotypes in inflammatory diseases of the human lung. *Eur Respir J* 2007; **175**:878–86.
- 11 Weinberger M, Hendeles L. Theophylline in asthma. *N Engl J Med* 1996; **334**:1380–8.
- 12 Barnes PJ. Theophylline. New perspectives for an old drug. *Am J Respir Crit Care Med* 2003; **167**:813–8.
- 13 Kraft M, Pak J, Borish L, Martin RJ. Theophylline's effect on neutrophil function and the late asthmatic response. *J Allergy Clin Immunol* 1996; **98**:251–7.
- 14 Yasui K, Agematsu K, Shinozaki K *et al.* Effects of theophylline on human eosinophil functions: comparative study with neutrophil functions. *J Leukoc Biol* 2000; **68**:194–200.
- 15 The American Lung Association Asthma Clinical Research Centers. Clinical trial of low-dose theophylline and montelukast in patients with poorly controlled asthma. *Am J Respir Crit Care Med* 2007; **175**:235–42.
- 16 Kidney J, Dominguez M, Taylor PM, Rose M, Chung KF, Barnes PJ. Immunomodulation by theophylline in asthma. Demonstration by withdrawal of therapy. *Am J Respir Crit Care Med* 1995; **151**:1907–14.
- 17 Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α . *J Exp Med* 1994; **179**:1109–18.
- 18 Yoneyama S, Kawai K, Tsuno NH *et al.* Epigallocatechin gallate affects human dendritic cell differentiation and maturation. *J Allergy Clin Immunol* 2008; **121**:209–14.
- 19 Addi AB, Lefort A, Hua X *et al.* Modulation of murine dendritic cell function by adenosine nucleotides and adenosine: involvement of the A2B receptor. *Eur J Immunol* 2008; **38**:1610–20.
- 20 Wilson JM, Ross WG, Agbai ON, Frazier R, Figler RA, Rieger J. The A2B adenosine receptor impairs the maturation and immunogenicity of dendritic cells. *J Immunol* 2009; **182**:4616–23.
- 21 Ongini E, Fredholm BB. Pharmacology of adenosine A2A receptors. *Trends Pharmacol Sci* 1996; **17**:364–72.
- 22 Ferrero D, Pessano S, Pagliardi GL, Rovera G. Induction of differentiation of human myeloid leukemias: surface changes probed with monoclonal antibodies. *Blood* 1983; **61**:171–9.
- 23 Collins SJ, Ruscetti FW, Gallagher RE, Gallo RC. Terminal differentiation of human promyelocytic leukemia cells induced by dimethylsulfoxide and other polar compounds. *Proc Natl Acad Sci USA* 1979; **75**:2458–62.
- 24 Setsukinai K, Urano Y, Kakinuma K, Majima HJ, Nagano T. Development of novel fluorescence probes that can reliably detect reactive oxygen species and distinguish specific species. *J Biol Chem* 2003; **278**:3170–5.
- 25 Parvathenani LK, Buescher ES, Chacon-Cruz E, Beebe SJ. Type Ic AMP-dependent protein kinase delays apoptosis in human neutrophils at a site upstream of caspase-3. *J Biol Chem* 1998; **273**:6736–43.
- 26 Feoktistov I, Polosa R, Holgate ST, Biaggioni I. Adenosine A2B receptor: a novel therapeutic target in asthma? *Trends Pharmacol Sci* 1998; **19**:148–53.
- 27 Jaffar ZH, Sullivan P, Page C, Costello J. Low-dose theophylline modulates T-lymphocyte activation in allergen-challenged asthmatics. *Eur Respir J* 1996; **9**:456–62.
- 28 Toyoshima K, Doi S, Murayama N. Effect of long-term administration of theophylline on serum immunoglobulin E level in asthmatic children, preliminary study. *Clin Exp Allergy* 1996; **26** (Suppl. 2):28–31.
- 29 Louis R, Bettiol J, Cataldo D, Sele J, Henquet M, Radermecker M. Effect of a 4-week treatment with theophylline on sputum eosinophilia and sputum eosinophils chemotactic activity in steroid-naïve asthmatics. *Clin Exp Allergy* 2000; **30**:1151–60.
- 30 Polson JB, Kazanowski JJ, Goldman AL, Szentivanyi A. Inhibition of human pulmonary phosphodiesterase activity by therapeutic levels of theophylline. *Clin Exp Pharmacol Physiol* 1978; **5**:535–9.
- 31 Berstrand H. Phosphodiesterase inhibition and theophylline. *Eur J Respir Dis* 1980; **61**:37–44.
- 32 Takuma K, Baba A, Matsuda T. Astrocyte apoptosis: implications for neuroprotection. *Prog Neurobiol* 2004; **72**:111–27.
- 33 Mirza A, Basso A, Black S *et al.* RNA interference targeting of A1 receptor-overexpressing breast carcinoma cells leads to diminished rates of cell proliferation and induction of apoptosis. *Cancer Biol Ther* 2005; **4**:1355–60.
- 34 D'Alimonte I, Ballerini P, Bargi E *et al.* Staurosporine-induced apoptosis in astrocytes is prevented by A1 adenosine receptor activation. *Neurosci Lett* 2007; **418**:66–71.
- 35 Salmon JE, Brogle N, Brownlie C *et al.* Human mononuclear phagocytes express adenosine A1 receptors. *J Immunol* 1993; **151**:2775–85.
- 36 Tsutsui S, Schnermann J, Noorbakhsh F *et al.* A1 adenosine receptor upregulation and activation attenuates neuroinflammation and demyelination in a model of multiple sclerosis. *J Neurosci* 2004; **24**:1521–9.
- 37 Hasko G, Pacher P, Deitch EA, Vizi ES. Shaping of monocyte and macrophage function by adenosine receptors. *Pharmacol Therapeut* 2007; **113**:264–75.
- 38 Arakawa H, Morikawa A, Shigeta M *et al.* Plasma theophylline concentrations and airway function in asthmatic children receiving standard and modified RTC therapy. *J Asthma* 1992; **29**:235–43.