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Effects of inhaled ciclesonide on circulating T-helper type 1/T-helper type 2 cells in atopic asthmatics after allergen challenge

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Summary

Background The predominance of T-helper type 2 (Th2) lymphocytes is thought to underlie the pathogenesis of asthma. Allergen inhalation challenge in atopic asthmatic subjects is associated with decreased interferon-γ (IFN-γ) positive $CD4^+$ and $CD8^+$ lymphocytes in peripheral blood and induced sputum.

Objective This study examined the effects of an inhaled corticosteroid on these previously described allergen-induced changes in circulating Th1 and Th2 lymphocytes.

Methods Subjects were randomized to 7 days of placebo, 40 or 80 μg ciclesonide in a crossover study. Airway responses and peripheral blood were measured before and after treatment, and 24 h after allergen challenge.

Results Ciclesonide 40 and 80 μg significantly attenuated the late response and sputum eosinophils at 8 h post-allergen ($P < 0.05$). Circulating IFN-γ positive $CD4^+$ lymphocytes decreased after allergen challenge with placebo ($P < 0.05$), and this was inhibited by 40 μg ciclesonide treatment ($P < 0.05$). There was no effect of allergen inhalation or ciclesonide on IL-4-positive $CD4^+$ lymphocytes or IFN-γ and IL-4-positive $CD8^{high}$ lymphocytes. The allergen-induced change of IFN-γ/IL-4 ratio on $CD4^+$ cells correlated with the allergen-induced change of peripheral blood eosinophils.

Conclusions The results of this study suggest that attenuation of allergen-induced airway responses by ciclesonide may be mediated through regulation of IFN-γ-positive $CD4^+$ cells.

Keywords airway inflammation, allergen challenge, atopic asthma, $CD4^+$ lymphocytes, $CD8^+$ lymphocytes, interferon-γ, interleukin-4, intracellular cytokines
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Introduction

Atopic asthma is a chronic disease characterized by airway and systemic inflammation. Elevated levels of inflammatory cells include activated eosinophils and T lymphocytes, which are further elevated in association with allergen-induced late asthmatic response [1–4]. There is increasing evidence that activated T lymphocytes play a central role in the pathogenesis of asthma through cytokine production. This is supported by studies showing increased activation of T lymphocytes following allergen provocation [5,6], and by studies showing an association between activated T lymphocytes and severity of asthma [7]. We have previously demonstrated a relationship between allergen-induced airway responses and cytokine expression of T lymphocytes sampled from airways and peripheral blood [8,9].

Activated $CD4^+$ T helper (Th) lymphocytes are subdivided into two distinct phenotypes based on the cytokines they produce [10]. Th1 lymphocytes produce cytokines such as interferon-γ (IFN-γ), interleukin (IL)-2 and IL-12, whereas Th2 lymphocytes produce cytokines such as IL-4, IL-5 and IL-13. Th1/Th2 counter-regulation has also been described, with each lymphocyte population capable of inhibiting and/or regulating the development and/or phenotype induced by the other [10]. Several studies have demonstrated an imbalance of Th1/Th2 towards Th2 predominance, which is thought to underlie asthmatic responses [11].

In the mouse model, there is evidence that IFN-γ produced from Th1 lymphocytes suppresses allergen-induced Th2 airway inflammation [12,13]. Furthermore, airway inflammation and hyper-responsiveness are

persistent in sensitized IFN-γ knockout or IFN-γ receptor knockout mouse models [14,15]. In atopic asthmatics, systemic and airway IFN-γ-producing T lymphocytes decrease significantly after allergen provocation [4,9], suggesting that airway and systemic inflammation may be allowed to skew towards Th2 during the late asthmatic response.

Inhaled corticosteroids (ICS) are the most effective prophylactic agents available for the treatment of asthma [16,17]. The effects of ICS on Th1/Th2 balance may occur through regulation of circulating Th1 (IFN-γ and $CD4^+$) and Th2 (IL-4 and $CD4^+$) lymphocytes, and/or levels of $CD8^+$ lymphocytes. Ciclesonide is an ICS under development for the treatment of asthma and allergic rhinitis. Inhaled ciclesonide reduced early and late asthmatic responses after allergen inhalation [18], and reduced allergen-induced eosinophilia [19] but it is not known whether these effects are related to steroid-induced effects on Th1/Th2 balance. To evaluate this hypothesis, we measured expression of IFN-γ and IL-4 from allergen-induced Th1 and Th2 lymphocytes in peripheral blood using a three-colour flow cytometric intracellular cytokine assay.

Methods

Subjects

Ten non-smoking subjects (seven male, 33 ± 4.2 years) with stable atopic asthma were selected for this study (Table 1). Each subject underwent a medical history, physical examination, and pulmonary function tests. Subjects had no asthma exacerbations or respiratory infections within the previous 4 weeks, and used only a short-acting inhaled β2-agonist for treatment of asthma, withheld for 8 h before each visit. All subjects had baseline forced expiratory volume in 1 s (FEV_1) greater than 70% predicted. Atopy was defined with a positive skin prick test (> 2 mm wheal) to at least one of 19 common allergens. Subjects underwent a screening period to document provocative concentration of methacholine, causing a 20% reduction in FEV_1 (PC_{20}) of less than 16 mg/mL, and allergen-induced early and late asthmatic responses of at least a 20% and 15% decline in FEV_1, respectively. The study was approved by the Hamilton Health Sciences Research Ethics Board, and informed consent was obtained from each subject.

Study design

This was a double-blind, placebo-controlled, randomized, three-way crossover study. After screening, subjects were randomized to one of six treatment sequences of placebo, 40 or 80 μg ciclesonide inhaled once daily. Treatment periods lasted 7 days and after a minimum washout period of 4 weeks, subjects received the next treatment, following a computer-generated random code provided by the study biostatistician. On the first morning of each treatment period, pre-dose measurements were carried out in the following order: peripheral blood was collected to determine the baseline level of circulating inflammatory cells including IFN-γ and IL-4-positive T lymphocytes, methacholine challenge was carried out to determine airway responsiveness and sputum was induced to determine airway inflammatory status. The first dose of the study drug was then administered in the laboratory. Subjects self-administered subsequent doses for the following six mornings immediately after waking. On the morning of day 5, blood sampling, methacholine PC_{20} and sputum induction were repeated. On the morning of day 6, allergen inhalation challenge was carried out and FEV_1 was measured until 8 h after challenge, and sputum was

Table 1. Subject characteristics during screening period

Subject no. ($n = 10$)	Age (years)	Sex	FEV_1 (%predicted)	PC_{20} (mg/mL)	Allergen/dilution/cumulative dose (BU)	EAR (%)	LAR (%)
50001	25	F	91.2	1.12	HDM/1 : 64/213	− 38.7	− 29.0
50002	21	M	114.6	8.95	HDM/1 : 16/853	− 26.5	− 18.4
50004	42	M	102.4	4.59	HDM/1 : 32/548	− 30.9	− 18.5
50005	20	M	74.1	0.31	HDM/1 : 1024/13	− 20.3	− 58.0
50008	26	F	87.6	1.62	RGW/1 : 16/1138	− 39.3	− 25.0
50011	54	M	101.2	0.88	CAT/1 : 32/142	− 47.8	− 17.9
50012	33	M	89.6	15.56	RGW/1 : 64/284	− 31.5	− 17.8
50013	54	M	71.2	3.41	HDM/1 : 64/213	− 28.0	− 20.8
50014	19	F	110.5	0.54	HDM/1 : 128/107	− 26.8	− 36.8
50015	39	M	77.9	5.15	HDM/1 : 128/107	− 29.6	− 16.7
Mean	33.3		92.0	2.22*		− 36.8	− 27.4
SEM	4.2		4.7	1.49		3.68	3.91

*Geometric mean and SEM.

FEV_1, forced expiratory volume in 1 sec; PC_{20}, provocative concentration of methacholine causing a 20% fall in FEV_1; HDM, house dust mite; BU, Biological units; EAR, early asthmatic response; LAR, late asthmatic response; M, male; F, female.

induced at 8 h post-allergen. On the morning of day 7 (24 h after allergen inhalation), blood was collected, and methacholine PC_{20} and sputum induction were repeated.

Laboratory procedures

Methacholine inhalation challenge. Methacholine inhalation challenge was performed as described by Cockcroft et al. [20]. Subjects inhaled doubling concentrations of nebulized methacholine chloride by tidal breathing for 2 min. The test was terminated when a 20% decline in FEV_1 occurred, and the PC_{20} was calculated.

Allergen inhalation challenge. Allergen inhalation challenge was performed as described by O'Byrne et al. [1]. The allergen extract was selected and diluted for inhalation at a concentration determined from the skin prick test results and the methacholine PC_{20} [21]. Doubling concentrations of allergen were nebulized and delivered by tidal breathing for 2 min. until a 20% decline in FEV_1 was measured. The FEV_1 was subsequently measured at regular intervals up to 8 h post inhalation. The early asthmatic response was the largest % decline in FEV_1 within 2 h, and the late asthmatic response was the largest percent decline in FEV_1 in the period beginning 3 h and ending 8 h after allergen inhalation.

Sputum analysis. Sputum was induced using the method of Pin et al. [22]. Subjects inhaled 3%, 4% and 5% saline for 7 min each and expectorated sputum after each inhalation period. Processing of sputum was carried out on selected portions using the method described by Pizzichini et al. [23]. Differential cell counts were obtained from the mean of two slides, and percent eosinophils was used as an index of allergen-induced airway inflammation.

Blood cell cultures. Total leukcocyte counts were calculated using a haemocytometer, and differential cell counts were obtained from blood smears. Cell populations were expressed as the number per millilitre blood. Peripheral blood mononuclear cells were isolated by Accu-Prep™ Lymphocyte (Accurate Chemical and Scientific Corp., Westbury, New York, USA) density gradient, re-suspended in RPMI-1640, and cultured in the presence of phorbol 12-myristate 13-acetate (PMA, Sigma, Oarkville, ON, Canada, 25 ng/mL), ionomycin (Sigma, 2 μM) and monensin (GolgiStop™, PharMingen San Diego, CA,USA; 0.2 μL/mL) for 4 h at 37 °C in 5% CO_2 and 85% humidity. Cell viability was assessed by the trypan blue exclusion method before and after incubation, being $> 99\%$ and $> 98\%$, respectively. Non-adherent cells were harvested and processed for staining.

Flow cytometric analysis. All monoclonal antibodies (mAb) were purchased from PharMingen . Phycoerythrin (PE)-conjugated anti-human IFN-γ mAb, B27 (mouse IgG_1), anti-human IL-4 mAb, 8D4-8 (mouse IgG_1) and isotype control mouse IgG_1 were used for flow cytometry. Fluorescent isothiocyanate (FITC)-conjugated anti-CD4 mAb, RPA-T4 (mouse IgG_1) and CyChrome™-conjugated anti-CD8 mAb, RPA-T8 (mouse IgG_1), were used for identifying each T lymphocyte subset.

A three-colour flow cytometric intracellular cytokine assay was performed as described previously [8,9]. Briefly, cells were stained with FITC-conjugated anti-CD4 mAb and CyChrome™-conjugated anti-CD8 mAb, fixed in 100 μL of fixation medium A (Caltag, Burlingame, CA, USA) containing 4% paraformaldehyde, resuspended in 100 μL of permeabilization medium B (Caltag) and then stained with PE-conjugated anti-IFN-γmAb, anti-IL-4 mAb or isotype control.

A FACScan flow cytometer (Becton Dickinson, San Jose, California USA) equipped with a 15-mA argon ion laser and filter settings for FITC (530 nm) (FL-1), PE (585 nm) (FL-2) and CyChrome™ (650 nm) (FL-3) was used, and the data were analysed with CELLQuest software (Becton Dickinson, Mississauga, Ontario, Canada). A gate was set on the lymphocyte population based on the forward and side scatter plot, and then analysed for the detection of $CD4^+$ or $CD8^+$-stained positive lymphocytes by FL-1 and FL-3. The lymphocytes staining positive for IFN-γ or IL-4 were analysed by means of detection of FL-2 on either FL-1 ($CD4^+$)-or FL-3 ($CD8^+$) lymphocytes (Fig. 1).

Statistical Analysis

SPSS for Windows, version 12 (SPSS Inc., Chicago, IL USA), was used to analyse the data. Data are shown as the mean ± standard error of the mean (SEM). Measurements of methacholine PC_{20} and sputum cell numbers were log transformed before analysis and are reported as geometric means and GSEM. Peripheral blood and sputum cells between placebo and ciclesonide were compared using two-factor repeated measures analysis of variance (ANOVA) to analyse the effects of treatment and time. Post hoc testing was performed using Duncan's test to assess for significant effects while controlling for multiple comparisons. All comparisons were two-tailed, and probability (P) values < 0.05 were considered significant. Correlations were determined by Spearman's rank correlation test.

Results

Both doses of ciclesonide had an effect on allergen-induced bronchoconstriction. The late response was significantly attenuated by both doses of ciclesonide

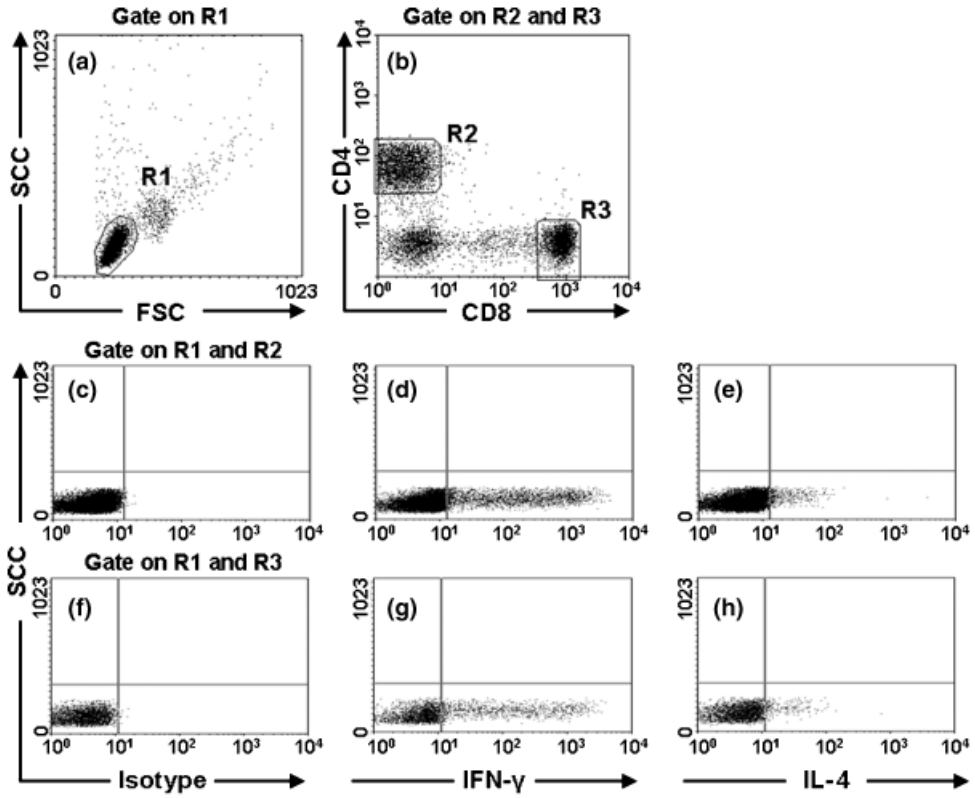

Fig. 1. Flow cytometry gating strategy for cells stained with fluorescein isothiocyanate FITC-CD4, CyChrome™-CD8 and PE-intracellular cytokines 4 h after incubation with PMA and ionomycin. (a): Lymphocyte population; (b): $CD4^+$ and $CD8^+$ lymphocytes; (c): $CD4^+$ isotype control; (d): $CD4^+$ IFN-γ positive; (e): $CD4^+$ IL-4 positive; (f): $CD8^+$ isotype control; (g): $CD8^+$ IFN-γ positive (h): $CD8^+$ IL-4 positive.

($P < 0.05$), whereas only 80 μg ciclesonide attenuated the allergen-induced early response ($P < 0.05$, Fig. 2).

The methacholine PC_{20} decreased significantly 24 h after allergen inhalation, from 3.02 mg/mL at day 5 to 1.66 mg/mL with placebo treatment, and from 3.63 mg/mL at day 5 to 2.04 mg/mL with 80 μg ciclesonide ($P < 0.05$).

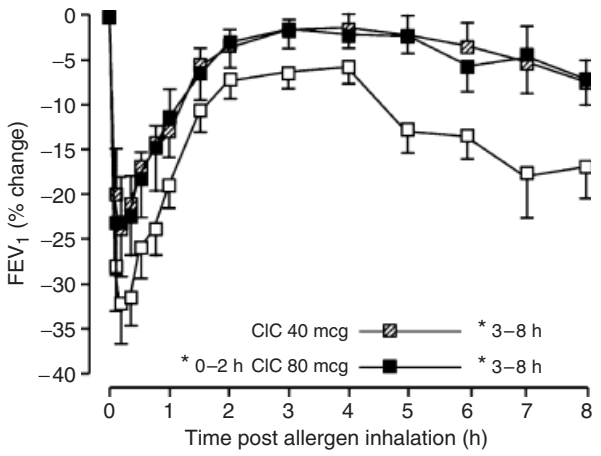

Fig. 2. Percent change in forced expiratory volume (FEV_1) (mean and standard error of the mean (SEM), following allergen inhalation with placebo (open squares), 40 μg ciclesonide (hatched squares) and 80 μg ciclesonide (closed squares) treatment. *$P < 0.05$ compared with placebo.

There was no significant allergen-induced decline in methacholine PC_{20} with 40 μg ciclesonide, being 3.16 mg/mL at day 5 and 2.63 mg/mL 24 h after allergen challenge ($P > 0.05$). The allergen-induced decrease in PC_{20} was not significantly different between placebo or ciclesonide treatments ($P > 0.05$). Although we were able to detect significant effects of allergen on methacholine PC_{20}, the study was not powered to detect the effect of ciclesonide on allergen-induced airway hyper-responsiveness.

The number of sputum and peripheral blood eosinophils measured at the pre-treatment baseline was not different between placebo and ciclesonide, nor was there a difference after 5 days of treatment ($P > 0.05$; Fig. 3). The number of sputum eosinophils increased significantly at 8 h ($P < 0.01$), and 24 h ($P < 0.05$) post-allergen challenge. Treatment with 40 and 80 μg ciclesonide significantly attenuated the allergen-induced increases in sputum eosinophils at 8 h ($P < 0.05$) but not at 24 h. The number of peripheral blood eosinophils increased 24 h after allergen ($P < 0.05$) with placebo and this increase was significantly attenuated by 80 μg ciclesonide ($P < 0.05$; Fig. 3).

The number of peripheral blood $CD4^+$ lymphocytes did not change following 5 days of ciclesonide treatment ($P > 0.05$; Fig. 4), but were lower 24 h post-allergen with 80 μg ciclesonide compared with pre-treatment levels

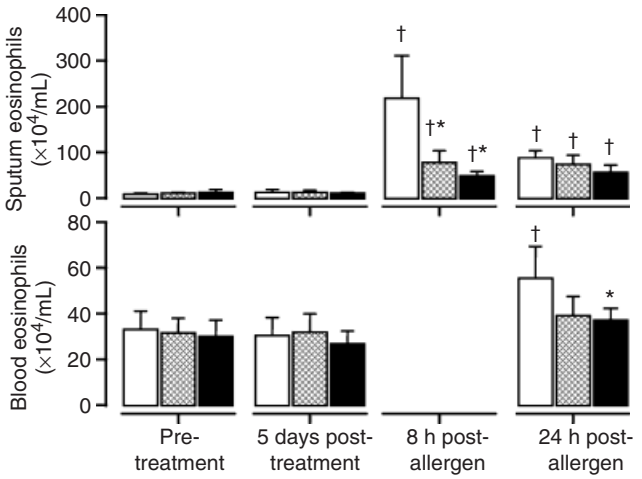

Fig. 3. Number of sputum (*top panel*) and peripheral blood (*bottom panel*) eosinophils in response to allergen inhalation challenge during treatment with placebo (*open bars*), 40 μg (*hatched bars*) and 80 μg ciclesonide (*closed bars*). †$P < 0.05$ compared with post-treatment (Day 5) in the same treatment group. *$P < 0.05$ compared with placebo at the same time point.

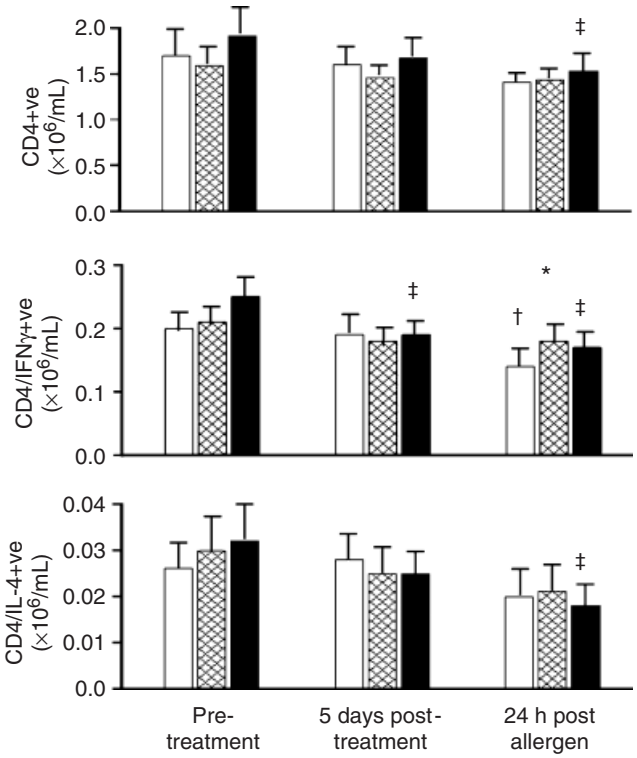

Fig. 4. Number of $CD4^+$ cells (*top panel*), interferon-γ (IFN-γ)-positive $CD4^+$ cells (center panel) and and IL-4-positive CD4+ cells (bottom panel) measured pre treatment, post-treatment (Day 5) and 24 h after allergen inhalation with placebo (open bars), 40 μg (hatched bars) and 80 μg ciclesonide (closed bars). ‡$P < 0.05$ compared with pre-treatment in the same treatment group. †$P < 0.05$ compared with post-treatment (day 5) in the same treatment group; *$P < 0.05$ compared with placebo at the same time point.

($P < 0.05$; Fig. 4). The same findings were observed in the IL-4-positive $CD4^+$ lymphocyte subset, which may be a reflection of the change in the level of $CD4^+$ lymphocytes (Fig. 4). Following placebo treatment, there was an allergen-induced decrease in the number of IFN-γ positive $CD4^+$ lymphocytes measured 24 h post-allergen compared with 5 days post placebo treatment ($P < 0.05$; Fig. 4). Following 80 μg ciclesonide treatment, there was a decrease in the number of IFN-γ-positive $CD4^+$ lymphocytes measured 5 days post-treatment and at 24 h post-allergen when compared with pre-treatment levels ($P < 0.05$, Fig. 4). It is not known whether the reduction measured at 24 h post-allergen is a result of a lower baseline number, or whether this is simply an effect of allergen challenge. The allergen-induced decrease in IFN-γ-positive $CD4^+$ lymphocytes was inhibited by 40 μg ciclesonide ($P < 0.05$), but not by 80 μg ciclesonide (Fig. 4). The discrepant results reflect insufficient powering of the study to make comparisons between the ciclesonide treatment groups.

The number of $CD8^{high}$ lymphocytes measured before treatment was unchanged after 5 days of treatment with placebo, 40 and 80 μg ciclesonide ($P > 0.05$). With placebo treatment, there was an allergen-induced decrease in the number of $CD8^{high}$ lymphocytes, declining from $0.70 \pm 0.11 \times 10^6$/mL post-treatment to $0.58 \pm 0.07 \times 10^6$/mL at 24 h post-allergen ($P < 0.05$). There was no change from pre-treatment levels after 5 days of treatment or at 24 h post-allergen ($P > 0.05$). With 40 and 80 μg ciclesonide, the number of $CD8^{high}$ lymphocytes did not change from pre-to-post treatment, or from post treatment to 24 h post allergen ($P > 0.05$), but declined from pre-treatment levels of 0.68 ± 0.09 and $0.68 \pm 0.08 \times 10^6$ mL, respectively to 0.56 ± 0.07 and $0.54 \pm 0.07 \times 10^6$/mL, respectively, at 24 h post-allergen ($P < 0.05$). With 80 μg ciclesonide treatment, the number of IFN-γ-positive $CD8^{high}$ lymphocytes decreased significantly from $0.23 \pm 0.03 \times 10^6$/mL pre-treatment to $0.17 \pm 0.02 \times 10^6$/mL at 24 h post-allergen ($P < 0.05$). This decline may be due to the overall decrease in $CD8^{high}$ lymphocytes observed with 80 μg ciclesonide at 24 h post-allergen. There were no effects of ciclesonide or allergen on the number of IL-4-positive $CD8^{high}$ lymphocytes, or CD4/CD8 ratio ($P < 0.05$, data not shown).

Discussion

Ciclesonide attenuated the maximum percent decline in FEV_1 during the late asthmatic response, as well as the allergen-induced increase of sputum and peripheral blood eosinophils, as shown previously [18,19]. This study also confirms previous findings that the level of circulating IFN-γ (Th1)-, but not IL-4 (Th2)-, positive $CD4^+$ cells lymphocytes, decreases 24 h after allergen inhalation in subjects with asthma who develop a late asthmatic

response [9]. This study has shown that when these subjects are pre-treated with 40 μg ciclesonide, there is inhibition of the allergen-induced decrease in circulating IFN-γ positive $CD4^+$ lymphocytes. Insufficient power and short duration of treatment may explain the absence of effect of other lymphocyte subsets and sputum eosinophils at 24 h after allergen.

We demonstrated that the allergen-induced change of the IFN-γ/IL-4 ratio of $CD4^+$ lymphocytes was associated with that of the absolute number of peripheral blood eosinophils. We conclude that even at low doses, ciclesonide may attenuate an allergen-induced shift in Th1/Th2 during the late asthmatic response by attenuating the allergen-induced decrease in peripheral blood IFN-γ positive $CD4^+$ lymphocytes.

Although the definitions of Th1 and Th2 lymphocyte phenotypes are clear in studies of mice, this distinction is less clear in humans. It is also known that the detection of these phenotypes is difficult without stimulation with agents such as phorbol ester and calcium ionophore, as human T cells can produce both IFN-γ and IL-4 after stimulation with these agents. The necessity to stimulate T cells with PMA and ionomycin makes it impossible to measure changes in intracellular cytokine levels induced by allergen and ciclesonide alone. However, the observation of consistent changes in intracellular cytokine staining following allergen challenge and ciclesonide treatment implies that there is a mechanism in addition to the PMA and ionomycin having an effect on the T cells' cytokine expression. The effect of phorbol ester and calcium ionophore on T cells has a similar effect to that of anti-CD3 stimulation [24]. Owing to our inability to detect IFN-γ- or IL-4-positive $CD4^+$ or $CD8^+$ cells without stimulation with PMA and ionomycin (data not shown), we elected to stimulate T cells in the current study despite the potential of introducing additional variables such as down-regulation of CD4 antigen by phorbol ester [25]. Preliminary experiments demonstrated no down-regulation of CD4 antigen surface expression with the concentration of PMA and ionomycin used in the current study.

IFN-γ can suppress IgE synthesis and the development of Th2 phenotype from Th0, naïve T cells [26] and is thought to down-regulate Th2-predominant asthmatic immune responses. Elevated levels of IFN-γ have been measured in serum, bronchoalveolar lavage (BAL) in other models of asthma [27,28] and increased levels of IFN-γ-producing T lymphocytes have been measured in BAL of subjects with asthma [29]. There is accumulating evidence suggesting that it may suppress allergen-induced inflammation. These observations suggest that IFN-γ may be up-regulated as a homeostatic mechanism to counteract the Th1/Th2 imbalance.

We observed an allergen-induced decrease in IFN-γ–positive $CD4^+$ lymphocytes in peripheral blood. Our results support the findings of Krug et al. [4], who have demonstrated that the IFN-γ staining BAL-derived $CD4^+$, $CD8^+$ and γδ T cells was decreased significantly in asthmatics but not control subjects after a segmental allergen challenge. Whether this subset of $CD4^+$ lymphocytes protect from or contribute to the allergen response is unknown. Increased post-allergen circulating levels with steroid treatment may lead to elevated levels migrating to the airways post-allergen. In this context, IFN-γ-positive $CD4^+$ lymphocytes could be acting in an anti-inflammatory capacity. On the other hand, elevated levels of IFN-γ-positive $CD4^+$ lymphocytes in the peripheral blood post-allergen may be due to the reduced efflux of these cells from the circulation to the airways. We were unable to obtain sufficient numbers of sputum cells to measure levels of IFN-γ positive $CD4^+$ lymphocytes in the airways; therefore, our data cannot indicate the potential role of these cells in the airway allergic response. Of interest, a recent study has shown that 67% of $CD4^+$ lymphocytes in BAL from steroid-naïve asthmatic patients were natural killer T (NKT) cells, and the proportion declines to 60% in asthmatic patients treated with corticosteroids [30]. These observations suggest that steroids may specifically reduce the level of these immunoregulatory NKT cells in the airways. We did not measure NKT cells in the current study; however, this is a topic of great interest in ours and other laboratories.

To investigate the effects of allergen inhalation challenge and inhaled ciclesonide on T lymphocyte cytokine production, we studied only atopic, mild asthmatics who demonstrated a dual airway response to allergen inhalation. We could demonstrate clearly that IFN-γ-positive $CD4^+$ lymphocytes decreased 24 h after allergen inhalation, but we could not detect a significant change in the IFN-γ positive $CD8^{high}$ lymphocytes, despite a report that the number of IFN-γ producing $CD8^+$ T lymphocytes is related to asthma severity, bronchial hyper-responsiveness and eosinophilia [31]. We may have found significant decreases in IFN-γ-producing $CD8^{high}$ lymphocytes had they been enumerated later than 24 h post-allergen [9]. This was not done, as it is well known that the peak airway inflammatory changes occur within 24 h after allergen inhalation [1,3].

We did not find an effect of allergen on circulating IL-4-positive $CD4^+$ and $CD8^{high}$ lymphocytes, nor did we observe a significant effect of ciclesonide when compared with placebo. Although IL-4-positive $CD4^+$ and $CD8^{high}$ lymphocytes were detected in all samples, the frequency was very low when compared with IFN-γ-positive lymphocytes. IL-4 can be secreted by a variety of non-T cells, including mast cells, eosinophils and basophils, and it is unclear whether T cells are a major source for IL-4 at inflammatory sites.

We measured IFN-γ- and IL-4-producing T lymphocytes, as the balance of Th1/Th2 cells may be a very important determinant of inflammatory status. Indeed, there was a significant negative correlation between the

allergen-induced circulating IFN-γ/IL-4 ratio and the allergen-induced number of peripheral blood eosinophils. There was no correlation with sputum eosinophils, which may be due to a difference in the kinetics of inflammation between airways and peripheral blood.

Corticosteroids have been shown to act directly on Th1 and Th2 lymphocytes, although the effects of ICS on T cells still remain unclear. *In vivo* treatment with oral prednisolone reduced the number of cells expressing mRNA for IL-4 and IL-5, and increased those expressing mRNA for IFN-γ in bronchial mucosa from asthmatics [32]. Brinkmann and Kristofic [33] have reported that a low concentration of corticosteroids suppressed Th2 cytokine production from $CD4^+$ memory T cells under chronic stimulation, whereas even high concentrations could not suppress IFN-γ production. This suggests that the sensitivity to corticosteroids might be different between Th1 and Th2 lymphocytes. In the current study, treatment with low doses of ciclesonide did not result in an allergen-induced decrease of IFN-γ-positive $CD4^+$ cells, in contrast to that observed with placebo treatment. Power calculations demonstrate that the number of subjects enroled was insufficient to observe significant differences in Th1/Th2 lymphocytes between placebo and ciclesonide treatment groups; however, these results suggest that *in vivo* ciclesonide may be able to modulate the cytokine production of $CD4^+$ cells to help restore the balance of Th1/Th2. In atopic asthmatics, the levels of most allergic inflammatory cells are strongly related between airway, peripheral blood and bone marrow compartments [34–36]. Unfortunately, in this study design, the number of T lymphocytes from induced sputum was not sufficient for flow cytometric intracellular cytokine assay. Further studies are therefore necessary using samples collected from BAL or bronchial biopsy, as several reports have shown that there are differences in cytokine production and activation between T cells collected from airways and peripheral blood [36,37].

In conclusion, we have demonstrated that 7 days of treatment with inhaled ciclesonide at 40 and 80 μg once daily attenuate the allergen-induced increase in sputum eosinophils and the late asthmatic response. This study provides evidence that inhaled corticosteroids may also regulate the allergen-induced levels of Th1 lymphocytes, although a larger study population will be needed to confirm these preliminary observations. Restoration of Th1/Th2 lymphocyte balance may be one of the anti-inflammatory mechanisms by which inhaled corticosteroids attenuate the late airways response in subjects with atopic asthma.

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