

Adding salmeterol to an inhaled corticosteroid reduces allergen-induced serum IL-5 and peripheral blood eosinophils

Julia G. Koopmans, MD,^a René Lutter, PhD,^{a,b} Henk M. Jansen, MD, PhD,^a
and Jaring S. van der Zee, MD, PhD^a Amsterdam, The Netherlands

Background: Adding a long-acting β_2 -agonist to inhaled corticosteroids results in better symptomatic asthma control than increasing the dose of inhaled corticosteroids.

Objective: Investigating whether adding the long-acting β_2 -agonist salmeterol to the inhaled corticosteroid fluticasone propionate has an effect on allergen-induced allergic inflammation in asthma.

Methods: Bronchial allergen challenges were performed in 26 patients with allergic asthma, pretreating them with a single dose of either fluticasone/salmeterol (100/50 μ g) or fluticasone alone (100 μ g), in a double-blind, randomized, cross-over design. Sputum and serum markers of bronchial inflammation were measured after allergen challenge, as well as lung function parameters. Primary outcomes were sputum eosinophil numbers and eosinophil cationic protein.

Results: Asthmatic responses after allergen challenge were significantly reduced after pretreatment with fluticasone/salmeterol relative to fluticasone alone. Sputum inflammatory markers after allergen challenge were not significantly affected by fluticasone/salmeterol pretreatment. By contrast, serum IL-5 was significantly reduced (geometric mean serum IL-5 [SEM]: 0.5 [0.3] vs 1.1 [0.3] pg/mL 1 hour and 0.6 [0.3] vs 1.1 [0.3] pg/mL 6 hours after challenge with fluticasone/salmeterol vs fluticasone alone pretreatment, respectively; P values < .05). Also, peripheral blood eosinophils were significantly reduced (geometric mean number $\times 10^6/L$ [SEM]: 172 [0.1] vs 237 [0.1] at 6 hours and 271 [0.1] vs 351 [0.1] at 24 hours with fluticasone/salmeterol vs fluticasone alone pretreatment, respectively; P < .05).

Conclusion: Adding salmeterol to fluticasone reduces allergen-induced serum IL-5 and peripheral blood eosinophils. This phenomenon may contribute to the improved clinical outcomes that result from adding a long-acting β_2 -agonist to inhaled corticosteroids. (J Allergy Clin Immunol 2005;116:1007-13.)

Key words: Asthma, inflammation, sputum, bronchodilator agents, anti-inflammatory agents

Inhaled corticosteroids (ICS) are recommended as first-line treatment for asthma.¹ Their use is associated with a reduction in inflammatory cells in the airways² as well as with an improvement in exacerbation frequencies, symptom scores, and lung function.³ Several large clinical studies have shown that adding a long-acting β_2 -agonist (LABA) to ICS leads to better symptomatic asthma control and lower exacerbation frequencies than increasing the dose of inhaled steroids.⁴ Because of the increased effectiveness of this add-on therapy with respect to symptomatic asthma control, it has been suggested that adding a LABA to ICS exerts an additional effect on bronchial inflammation, complementary to the effect of ICS.⁵

There is evidence that β_2 -adrenergic receptors occur not only in airway smooth muscle cells but also on other resident and infiltrating inflammatory cells implicated in the underlying pathophysiologic process in asthma. The β_2 -adrenergic system may suppress inflammatory activity by activation of the adenylyl cyclase system and a subsequent rise in intracellular cyclic adenosine monophosphate.⁵ *In vitro* studies with both short-acting β -agonists and LABAs point to inhibitory effects on inflammatory cell activation and mediator release, cell adhesion and chemotaxis, and cell survival.⁶ In addition, there is some evidence that LABAs potentiate the effect of corticosteroids,⁷ possibly by ligand-independent activation of the glucocorticoid receptor.⁸ This subsequently may promote the translocation of glucocorticoid receptors into the nucleus, where they may influence the transcription of pro-inflammatory genes.⁹ One clinical study showed lower numbers of submucosal mast cells after 12 weeks of fluticasone/salmeterol compared with ICS monotherapy,¹⁰ although there was no effect on numbers of eosinophils. Another study did show lower numbers of eosinophils, as measured in bronchial biopsies, after adding salmeterol to beclomethasone dipropionate or budesonide.¹¹ Collectively, these findings suggest an anti-inflammatory effect of adding LABA therapy to ICS, although the precise underlying mechanisms remain to be elucidated.

Repeated allergen exposure is likely to play an important role in the persistence of allergic inflammation in

From ^athe Department of Pulmonology and ^bthe Laboratory of Experimental Immunology, Academic Medical Center, University of Amsterdam.

Disclosure of potential conflict of interest: The Department of Pulmonology received research grants from GlaxoSmithKline, AstraZeneca, and Actelion. Supported by GlaxoSmithKline. The contributing authors have no financial relationship with a commercial entity that has an interest in the subject of the manuscript.

Received for publication February 24, 2005; revised July 5, 2005; accepted for publication August 9, 2005.

Available online October 3, 2005.

Reprint requests: Jaring S. van der Zee, MD, PhD, Academic Medical Center, Department of Pulmonology, F4-208, PO Box 22700, 1100 DE Amsterdam, The Netherlands. E-mail: j.s.vanderzee@amc.uva.nl.

0091-6749/\$30.00

© 2005 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2005.08.016

Abbreviations used

A2M:	α 2-Macroglobulin
AUC:	Area under the curve
EAR:	Early asthmatic reaction
ECP:	Eosinophil cationic protein
ICS:	Inhaled corticosteroids
LABA:	Long-acting β_2 -agonist
LAR:	Late asthmatic reaction
RCE:	Relative coefficient of excretion

asthma.^{12,13} As yet, no studies have investigated the effect of adding a LABA to ICS on allergen-induced bronchial inflammation. In the current study, we investigated whether adding the long-acting β_2 -agonist salmeterol to the inhaled corticosteroid fluticasone propionate has an effect on allergen-induced allergic inflammation in asthma. Twenty-six patients with allergy and intermittent to mild persistent asthma¹ inhaled either fluticasone/salmeterol (100/50 μ g) or fluticasone alone (100 μ g) 20 minutes before a bronchial allergen challenge in a double-blind, randomized, 2-armed, cross-over design. Primary outcomes were the number of eosinophils and the eosinophil cationic protein (ECP) concentration in induced sputum. Serum IL-5, total eosinophil counts in peripheral blood, and sputum neutrophil-associated parameters were chosen as secondary outcomes. Furthermore, FEV₁ and bronchial hyperresponsiveness to histamine (PC₂₀histamine) measurements were performed both before and after the allergen challenges. Outcomes were measured at 1 (serum only), 6, and 24 hours after the allergen challenges.

METHODS

Patients

We recruited 26 nonsmoking patients with allergy and intermittent to mild persistent asthma,¹ characterized by a history of episodic wheeze and shortness of breath (Table I). The patients were sensitized to house dust mite (*Dermatophagoides pteronyssinus*, Der p) and/or cat dander, as determined by skin prick test. FEV₁ at baseline was at least 70% of the predicted value, and baseline bronchial hyperresponsiveness to histamine (PC₂₀histamine) was between 0.25 and 8.0 mg/mL. Patients had no exacerbations requiring oral steroids or antibiotics for 3 months before the study and no lower respiratory tract infection for 4 weeks before the study. Inhaled corticosteroids, antibiotics, theophylline, sodium cromoglycate, nedocromil sodium, or antileukotrienes were not allowed 4 weeks before and during the study. There was no comorbidity likely to interfere with the study. Patients were recruited via advertisement and via the Outpatient Department of Pulmonology of the Academic Medical Center in Amsterdam, The Netherlands. The Medical Ethical Committee of the Academic Medical Center approved the study, and all subjects gave written informed consent.

Study design

This was a double-blind, 2-armed, randomized, cross-over study (Fig 1). The study was preceded by 2 screening visits during which a full medical history, physical examination, skin prick tests, and lung

TABLE I. Characteristics of randomized patients at screening

Number of patients	26
Age,* y	24 (19, 35)
M/F	9/17
Total IgE,† IU/mL	213 (28, 8600)
SpecIgE,† IU/mL	26 (0.4, 590)
PD ₂₀ allergen,† BU	137 (14, 4960)
PC ₂₀ histamine,† mg/mL	1.4 (0.4, 7.9)
FEV ₁ ,‡ % predicted	101 (11.0)
EAR*§	-23 (-40, -20)
LAR*§	-18 (-52, 0)

BU, Biological units; SpecIgE, serum level of specific IgE directed against the type of allergen that was used during allergen challenge.

Values are expressed as *median (range), †geometric mean (range), or ‡mean (SD).

§Maximal change in FEV₁ during the first hour postchallenge (EAR) and from 1 to 10 hours postchallenge (LAR).

function measurements were performed to ensure eligibility for the study. In addition, outcomes were measured before and 24 hours after a screening bronchial allergen challenge. This screening bronchial allergen challenge was performed according to standardized procedures with purified allergen extracts of Der p or cat dander allergen (ALK-Abelló, Nieuwegein, The Netherlands) to determine the amount of allergen causing a 20% decline in FEV₁ during the early asthmatic reaction (PD₂₀allergen). During the study period, eligible patients subsequently underwent 2 allergen challenges with a fixed dose of allergen equal to the calculated PD₂₀allergen. Twenty minutes before the study allergen challenges, patients randomly inhaled either a single dose of fluticasone (100 μ g) in combination with salmeterol (50 μ g) or a single dose of fluticasone alone (100 μ g) in a cross-over design. All allergen challenges were followed by a washout period of 3 to 4 weeks. Outcomes were measured 24 hours prechallenge and 6 and 24 hours and 1 week postallergen challenge. An additional blood sample was taken at 1 hour postchallenge. Diurnal change in FEV₁ was determined on the day before allergen challenge. Patients were provided with ipratropium bromide (20 μ g) for relief of symptoms after the allergen challenges (during the first 24 hours postallergen challenge) and with salbutamol (200 μ g) for relief of symptoms on the remaining days of the study period. Ipratropium bromide usage after allergen challenge was recorded. Before every visit, patients abstained from rescue salbutamol for 8 hours. All drugs were administered via a dry powder inhaler (Diskus; GlaxoSmithKline, Zeist, The Netherlands) except for ipratropium bromide (Boehringer, Alkmaar, The Netherlands), which was administered via a metered dose inhaler.

Procedures

Spirometry, PC₂₀histamine, and bronchial allergen challenge were performed according to standardized procedures.¹⁴ For fixed dose allergen challenges, a single volume of relevant allergen was diluted to make up the PD₂₀allergen. The change in FEV₁ during the first hour after allergen challenge and the change in FEV₁ for 1 to 10 hours after allergen challenge were defined as the early asthmatic reaction (EAR) and the late asthmatic reaction (LAR) and expressed as the percentage change from baseline, as measured with the corresponding spirometer, dry rolling-seal (Sensor Medics BV, Bithoven, The Netherlands) and portable spirometer (Micromedical diarycard; Sensor Medics BV), respectively. The late asthmatic reaction was corrected for diurnal change in FEV₁ as determined on the day before the allergen challenge. The area under the curve (AUC) was

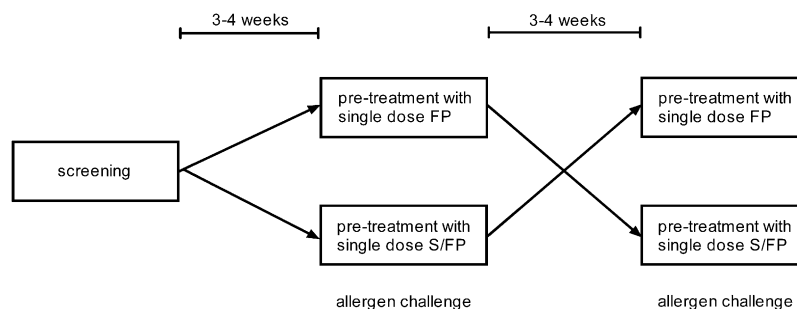


FIG 1. Schematic representation of study design. FP, Fluticasone propionate 100 µg; S, salmeterol 50 µg.

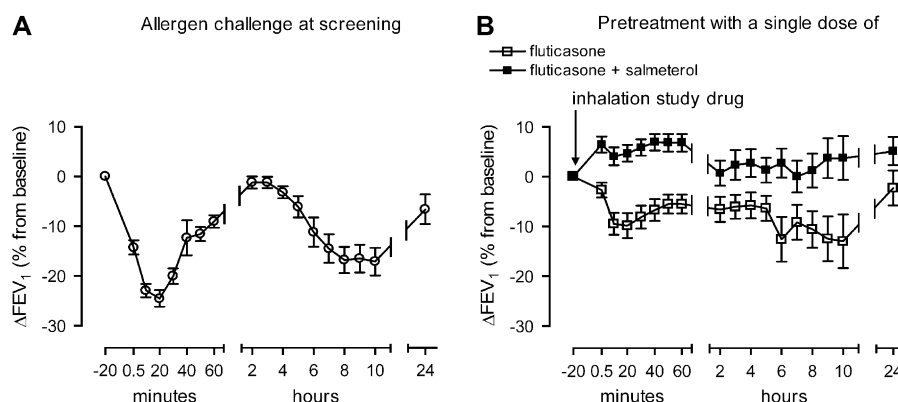


FIG 2. FEV₁ after the allergen challenge at screening (**A**) and after the study challenges (**B**) pretreated with either fluticasone propionate 100 µg or salmeterol 50 µg. Geometric mean values (SEM) are shown. BL, Baseline.

calculated from 0.5 to 60 minutes for the EAR (AUC_{EAR}) and from 1 to 10 hours for the LAR (AUC_{LAR}). Levels of total and specific IgE were determined in serum¹⁵ at the first screening visit. Sputum induction and processing of whole sputum samples were performed according to the method described by Fahy et al¹⁶ with minor modifications as described previously.¹⁷ Differential cell counts were expressed as percentage of cells, excluding squamous epithelial cells. Sputum samples containing more than 80% squamous cells on differential cell counting were excluded from analysis. Levels of ECP,¹⁸ myeloperoxidase,¹⁹ and IL-8 were measured in sputum and IL-5 in serum²⁰ with an ELISA, as described. IL-8 was quantitated by using the antibody pair from R&D Systems (Abingdon, United Kingdom; MAB 208 and BAF 208) following the manufacturer's instructions. Size selectivity of the blood-airway lumen barrier was determined by the relative coefficient of excretion (RCE),²¹ which is the ratio of sputum levels of α₂-macroglobulin (A2M) over that of albumin. A2M is 11-fold larger than albumin, and thus systemic leakage of A2M across the respiratory membrane is relatively reduced compared with that of the smaller albumin, except when there is loss of size selectivity. A2M was determined by ELISA²¹ and albumin by immunoturbidimetry (BN ProPec; Dade Behring, Marburg, Germany). All sputum assays were validated for the presence of dithiothreitol, by using a standard curve in dithiothreitol and by testing recovery of exogenously added marker protein to dithiothreitol-treated sputum samples. For most assays, samples were diluted 50-fold to rule out interference of dithiothreitol. SAS (version 8.2; SAS Institute Inc, Cary, NC) was used for statistical analyses. Prechallenge values were determined by dividing the values measured before the fixed dose allergen challenge by the number of valid cases at these time points. A mixed model ANOVA, accounting for period and time point, was used to determine

differences in postchallenge values between the 2 treatment groups. The estimation and testing procedure was a restricted maximum likelihood method so that a possible influence of missing values was corrected for. Missing values were considered to occur at random. Positively skewed outcomes were analyzed after logarithmic transformation. All *P* values are 2-tailed, and *P* levels of less than .05 were considered significant.

RESULTS

Screening

Twenty-six patients were randomized in this study (Table I). All patients exhibited an EAR of at least 20% from baseline after the screening allergen challenge (Fig 2, A). Elevated levels of inflammatory outcomes after the screening allergen challenge relative to levels before the screening allergen challenge were found (levels pre-screening and postscreening allergen challenge were measured in a subgroup of 14 patients; Table II).

Study allergen challenges: pretreatment with either fluticasone/salmeterol or fluticasone

Lung function parameters. A significant early-phase and late-phase drop in FEV₁ was still detected after pretreatment with fluticasone (Fig 2, B). The EAR was significantly reduced after pretreatment with fluticasone/salmeterol compared with pretreatment with fluticasone

TABLE II. Inflammatory parameters before and after screening allergen challenge*

	Prescreening allergen challenge	24 h Postscreening allergen challenge	P value
Sputum eosinophils†	1.2 (1.7)	4.5 (2.1)	.01
Sputum ECP‡	55.0 (1.5)	148 (1.8)	.04
Blood eosinophils§	187 (1.1)	373 (1.3)	.004
Serum IL-5	0.7 (1.3)	5.9 (1.7)	0.0005
Sputum neutrophils†	18 (1.4)	32 (1.6)	>.1
Sputum IL-8‡	202 (1.3)	317 (1.6)	>.1
Sputum myeloperoxidase‡	811 (1.3)	1673 (1.3)	.05
Sputum albumin¶	17 (1.5)	39 (1.3)	>.1
Sputum A2M‡	1278 (1.3)	1707 (1.4)	>.1
Sputum RCE#	75 (1.4)	44 (1.2)	>.1

*Values are expressed as geometric mean (SEM).

†Number $\times 10^4/\text{g}$.

‡ng/mL.

§Number $\times 10^6/\text{L}$.

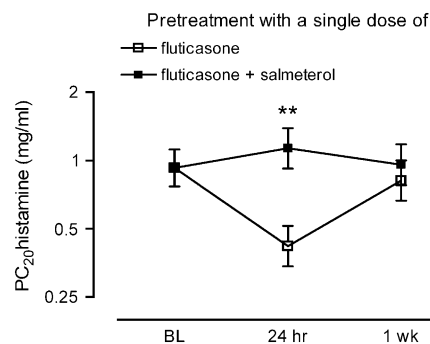
||pg/mL.

¶µg/mL.

#RCE $\times 10^3$.

alone (mean AUC from 0.5 to 60 minutes after challenge [SE]: 4.4 [2.1] after fluticasone/salmeterol pretreatment vs -8.9 [2.1] after fluticasone alone; $P < .0001$; Fig 2, B). Likewise, the LAR was significantly reduced after pretreatment with fluticasone/salmeterol compared with pretreatment with fluticasone alone (mean AUC from 1 to 10 hours [SE]: -1.1 [2.7] after fluticasone/salmeterol vs -12.1 [2.5] after fluticasone alone; $P = .001$). PC₂₀histamine was significantly higher 24 hours after allergen challenge with fluticasone/salmeterol pretreatment compared with fluticasone alone ($P < .0001$; Fig 3).

Inflammatory parameters. At 6 hours after allergen challenge, there was a significant decrease in A2M and albumin after both treatments compatible with vascular leakage. Twenty-four hours after allergen challenge, a significant increase in sputum eosinophils and ECP was demonstrated after pretreatment with fluticasone and in ECP only after fluticasone/salmeterol pretreatment (Table III). However, there were no significant differences between the 2 treatment groups in sputum eosinophils, ECP, neutrophils, IL-8, myeloperoxidase, A2M, albumin, and the RCE in induced sputum (Table III). Serum IL-5 was elevated at 1, 6, and 24 hours after challenge after fluticasone pretreatment but only at 24 hours after fluticasone/salmeterol pretreatment. Significantly lower concentrations of serum IL-5 were observed at 1 and 6 hours after allergen challenge with fluticasone/salmeterol pretreatment relative to fluticasone alone (geometric mean serum IL-5 [SEM]: 0.5 [0.3] vs 1.1 [0.3] pg/mL at 1 hour after challenge, and 0.6 [0.3] vs 1.1 [0.3] pg/mL at 6 hours after challenge with fluticasone/salmeterol and fluticasone alone pretreatment, respectively; P values $< .05$; Fig 4, A). Blood eosinophils showed a significant decrease at 1 and 6 hours after fluticasone/salmeterol pretreatment (Fig 4, B). In contrast, there was a significant increase at

**FIG 3.** PC₂₀histamine before and after allergen challenge pretreated with either fluticasone propionate 100 µg or salmeterol 50 µg. ** $P < .0001$. BL, Baseline.**TABLE III.** Sputum parameters after allergen challenge after pretreatment with fluticasone + salmeterol versus fluticasone alone*

	S/FP 6 h	FP¶ 6 h	S/FP 24 h	FP¶ 24 h
Eosinophils†	1.3 (1.5)	2.0 (1.5)	2.8 (1.5)	4.2 (1.6)#
ECP‡	46 (1.3)	66 (1.3)	99 (1.3)#	78 (1.3)#
Neutrophils†	16 (1.4)	21 (1.4)	27 (1.4)	17 (1.4)
IL-8‡	190 (1.4)	163 (1.4)	348 (1.4)	252 (1.4)
Myeloperoxidase‡	1443 (1.2)	1042 (1.2)	1316 (1.2)	1390 (1.3)
Albumin§	16 (1.4)#	14 (1.4)#	25 (1.4)	19 (1.4)
A2M‡	861 (1.3)#	627 (1.3)#	1297 (1.3)	1035 (1.3)
RCE	33 (1.3)	47 (1.3)	49 (1.2)	61 (1.3)

FP, Fluticasone propionate; S, salmeterol.

*Values are expressed as geometric mean (standard error of the mean).

†Number of cells $\times 10^4/\text{g}$ sputum.

‡ng/mL sputum.

§µg/mL sputum.

||RCE $\times 10^3$.¶S/FP vs FP: P values $> .2$.# P values $< .05$ for change from their respective prechallenge baseline values.

24 hours after fluticasone pretreatment, with unchanged values at time points 1 and 6 hours. Total eosinophil counts in peripheral blood at 6 and 24 hours after allergen challenge differed significantly between fluticasone/salmeterol and fluticasone pretreatment (geometric mean total eosinophil count in peripheral blood [SEM]: 172 [0.1] vs 237 [0.1] $\times 10^6/\text{L}$ at 6 hours after challenge, and 272 [0.1] vs 351 [0.1] $\times 10^6/\text{L}$ at 24 hours after allergen challenge with fluticasone/salmeterol and fluticasone pretreatment, respectively; P values $< .05$; Fig 4, B).

DISCUSSION

In this study, we have shown that serum IL-5 concentrations as well as total eosinophil counts in peripheral blood are significantly reduced after pretreatment with a single dose of salmeterol in a combination inhaler with

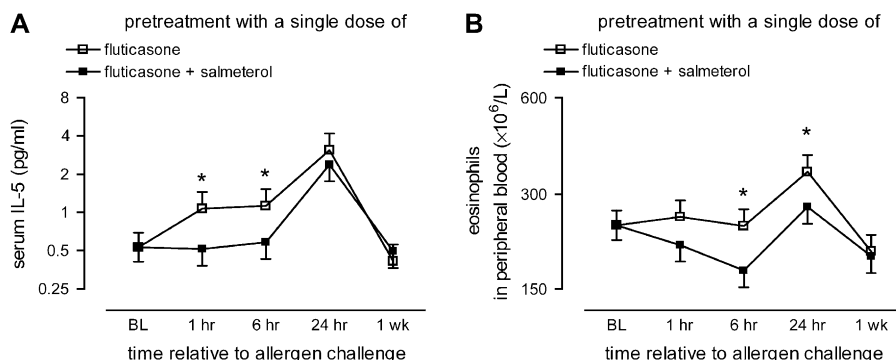


FIG 4. Levels of serum IL-5 (**A**) and peripheral blood eosinophils (**B**) after allergen challenge after pretreatment with fluticasone propionate 100 μ g or salmeterol 50 μ g. Geometric mean values (SEM) are shown. * $P < .05$. BL, Baseline.

fluticasone relative to a single dose of fluticasone alone. The differences in serum IL-5 were found as early as 1 and 6 hours after allergen challenge, whereas differences in total eosinophil counts in peripheral blood were most pronounced at 6 and 24 hours postchallenge. Sputum markers of bronchial inflammation were not affected in addition by fluticasone/salmeterol pretreatment. In concordance with earlier studies, the EAR and LAR as well as bronchial hyperresponsiveness after allergen challenge were significantly reduced by pretreatment with salmeterol.²² Our findings confirm and extend previous studies showing that the addition of a LABA to ICS may have an additional effect *in vivo* on allergic inflammation in patients with asthma that is complementary to the effect of ICS.

A prerequisite for establishing modulation of the allergen-induced changes by addition of salmeterol, as we aimed in this study, is incomplete blunting of the responses in outcome parameters by the dose of ICS. This criterion was met, because significant allergen-induced increases in inflammatory markers in sputum and blood and changes in physiological parameters were still detected after fluticasone treatment. The design of the study, however, does not permit estimation of separate ICS and LABA effects, because a formal placebo arm is lacking.

In patients with allergic asthma, IL-5 is considered to be mainly produced by T_H2 cells, fibroblasts, and mast cells. Thus, the differences in serum IL-5 after allergen challenge in the current study are likely explained by an effect of adding salmeterol to fluticasone on these cells. T_H2 cells may release IL-5 after antigen stimulation via several pathways. Activation of a T cell will lead to increased transcription of the IL-5 gene. Glucocorticosteroids are generally considered to influence the transcription of inflammatory genes, forming the basis of their anti-inflammatory properties in asthma. In addition, as mentioned, salmeterol may enhance the anti-inflammatory effects of glucocorticosteroids, possibly by ligand-independent activation of the glucocorticoid receptor.⁸ However, in the current study, the differences in serum IL-5 were already present at 1 hour after allergen challenge, whereas the process of T-cell activation with subsequent synthesis and

release of IL-5 is considered to take at least a few hours.²³ By contrast, the process of mast cell degranulation on cross-linking of IgE is considered to take a few minutes only. This is illustrated by the early decline in lung function shortly after allergen exposure, which is generally considered to be the result of mast cell activation²⁴ with subsequent release of bronchoconstrictor mediators like leukotrienes and histamine, which are stored in the mast cell granules.

It has been demonstrated that mast cells obtained from bronchial mucosa biopsies from patients with allergic asthma can synthesize and store cytokines that are considered important in the chronic inflammatory response in asthma, such as IL-4, IL-5, IL-6, IL-8, IL-13 and TNF- α .²⁵ Mast cells contain preformed cytokines, stored in their granules,²⁶ whereas T cells do not have the capacity to store cytokines like IL-5. In the bronchial mucosa, IL-5 appeared to be mainly present in tryptase-positive mast cells, whereas skin mast cells contained very little staining for IL-5.²⁶ Salmeterol has previously been shown to suppress mediator release from human lung *in vitro*.²⁷ In addition, salmeterol inhibits the release of TNF- α by human mast cells *in vitro*.²⁸ The inhibitory effect of β -agonists on allergen-induced skin reactions is well established.²⁹ Collectively, these findings suggest that the differences in serum IL-5 immediately after allergen challenge in the current study may be explained by an inhibitory effect on mast cell degranulation of adding salmeterol to fluticasone *in vivo* in patients with allergic asthma. To our knowledge, this is the first study to demonstrate *in vivo* in patients with asthma a reducing effect on serum IL-5 from adding salmeterol to fluticasone.

The importance of IL-5 in eosinophilic airway inflammation is illustrated by several studies.³⁰⁻³² Sensitization of T-cell-deficient, athymic mice was unable to induce eosinophilic airway inflammation or increase airway hyperresponsiveness unless IL-5 production was restored by systemic IL-5 treatment.³⁰ In patients, the development of airway eosinophilia is associated with increased IL-5 expression in the airway mucosa and with elevated concentrations of IL-5 in luminal fluid³¹ and serum.³² IL-5 is considered essential for the differentiation of eosinophil

precursors and also activates and prolongs survival of the mature cells in the tissue.³³ Leckie et al³⁴ found in patients with asthma that treatment with a single dose of anti-IL-5 significantly reduced blood and sputum eosinophil counts. There was however, no effect on either the EAR or LAR or on bronchial hyperresponsiveness in this study.³⁴ This finding may, at least partly, be explained by a differential effect of anti-IL-5 treatment on eosinophil counts in different body compartments.³⁵ After multiple dosing with anti-IL-5 (mepolizumab), the authors report that there was a 100% median decrease in eosinophil counts in peripheral blood, whereas there was only a 55% decrease in the bronchial mucosa.³⁵ Thus, anti-IL-5 may be less effective at depleting tissue eosinophils, which is in agreement with the fact that in the current study, we did not find significantly lower levels of sputum eosinophils and sputum ECP, in contrast with the reduced eosinophil counts in peripheral blood after allergen challenge. More recently, it has been evidenced that the function of eosinophils present in the airways may be IL-5-independent.³⁶ The main functions of IL-5 may be eosinophil hemopoiesis and release from the bone marrow, which is in line with the observed differences in eosinophil counts in peripheral blood, which appeared to occur at a later time point compared with the differences in serum IL-5. The early decrease in blood eosinophils in the absence of an early increase in serum IL-5 after fluticasone/salmeterol is compatible with the hypothesis that early eosinophil recruitment from the blood to the airways is less affected than recruitment of eosinophils from the bone marrow to the blood.

Alternatively, the discrepancy between serum and peripheral blood parameters on the one hand and sputum parameters on the other may be explained by a relatively lower power of the study with respect to sputum parameters. Sputum eosinophil numbers and ECP show relatively large intraindividual variation because of variable dilution by saliva and probably also because of inhomogeneous distribution of airway inflammation.¹⁶ Moreover, valid sputum samples are obtained in about 70% to 75% of the cases. Nevertheless, some studies claimed that sputum markers may be more sensitive than blood when assessing the anti-inflammatory response in asthma.³⁷

In conclusion, this is the first study to investigate the effects on allergen-induced bronchial inflammation from adding salmeterol to fluticasone in patients with asthma. We have shown that a single dose of salmeterol in a combination inhaler with fluticasone has an additional effect on serum IL-5 concentrations and eosinophil counts in peripheral blood after allergen challenge *in vivo* in patients with allergic asthma, complementary to the effect of a single dose of fluticasone alone. These effects are most likely explained by a stabilizing effect on mast cells. Thus, adding salmeterol to ICS may affect acute as well as chronic components of asthmatic inflammation, possibly contributing to improved asthma control.

We thank Barbara Smids-Dierdorp, Tamara Dekker, and Marianne van de Pol for their work on the inflammatory parameters in this study.

REFERENCES

1. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention: NHLBI/WHO workshop report. Bethesda (MD): National Institutes of Health, National Heart Lung and Blood Institute; 2002;NIH Publication No. 02-3659.
2. Schleimer RP, Schulman ES, MacGlashan DW Jr, Peters SP, Hayes EC, Adams GK III, et al. Effects of dexamethasone on mediator release from human lung fragments and purified human lung mast cells. *J Clin Invest* 1983;71:1830-5.
3. Dompeling E, van Schayck CP, van Grunsven PM, van Herwaarden CL, Akkermans R, Molema J, et al. Slowing the deterioration of asthma and chronic obstructive pulmonary disease observed during bronchodilator therapy by adding inhaled corticosteroids: a 4-year prospective study. *Ann Intern Med* 1993;118:770-8.
4. Shrewsbury S, Pyke S, Britton M. Meta-analysis of increased dose of inhaled steroid or addition of salmeterol in symptomatic asthma (MIASMA). *BMJ* 2000;320:1368-73.
5. Taylor DR, Hancox RJ. Interactions between corticosteroids and beta agonists. *Thorax* 2000;55:595-602.
6. Johnson M. Effects of beta2-agonists on resident and infiltrating inflammatory cells. *J Allergy Clin Immunol* 2002;110:S282-90.
7. Pace E, Gagliardo R, Melis M, La Grutta S, Ferraro M, Siena L, et al. Synergistic effects of fluticasone propionate and salmeterol on in vitro T-cell activation and apoptosis in asthma. *J Allergy Clin Immunol* 2004;114:1216-23.
8. Eickelberg O, Roth M, Lox R, Bruce V, Rudiger J, Johnson M, et al. Ligand-independent activation of the glucocorticoid receptor by beta2-adrenergic receptor agonists in primary human lung fibroblasts and vascular smooth muscle cells. *J Biol Chem* 1999;274:1005-10.
9. Barnes PJ, Pedersen S, Busse WW. Efficacy and safety of inhaled corticosteroids: new developments. *Am J Respir Crit Care Med* 1998;157:S1-53.
10. Wallin A, Sue-Chu M, Bjerner L, Ward J, Sandstrom T, Lindberg A, et al. Effect of inhaled fluticasone with and without salmeterol on airway inflammation in asthma. *J Allergy Clin Immunol* 2003;112:72-8.
11. Li X, Ward C, Thien F, Bish R, Bamford T, Bao X, et al. An antiinflammatory effect of salmeterol, a long-acting beta(2) agonist, assessed in airway biopsies and bronchoalveolar lavage in asthma. *Am J Respir Crit Care Med* 1999;160:1493-9.
12. Babu KS, Arshad SH. The role of allergy in the development of airway inflammation in children. *Paediatr Respir Rev* 2003;4:40-6.
13. Platts-Mills TA, Carter MC. Asthma and indoor exposure to allergens. *N Engl J Med* 1997;336:1382-4.
14. Sterk PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD, et al. Airway responsiveness: standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl* 1993;16:53-83.
15. Wittman AM, Stapel SO, Perdok GJ, Sjamsoedin DH, Jansen HM, Aalberse RC, et al. The relationship between RAST and skin test results in patients with asthma or rhinitis: a quantitative study with purified major allergens. *J Allergy Clin Immunol* 1996;97:16-25.
16. Fahy JV, Boushey HA, Lazarus SC, Mauger EA, Cherniack RM, Chinchilli VM, et al. NHLBI Asthma Clinical Network. Safety and reproducibility of sputum induction in asthmatic subjects in a multicenter study. *Am J Respir Crit Care Med* 2001;163:1470-5.
17. Nocker RE, Out TA, Weller FR, de Riemer MJ, Jansen HM, van der Zee JS. Induced sputum and bronchoalveolar lavage as tools for evaluating the effects of inhaled corticosteroids in patients with asthma. *J Lab Clin Med* 2000;136:39-49.
18. Reimert CM, Venge P, Kharazmi A, Bendtsen K. Detection of eosinophil cationic protein (ECP) by an enzyme-linked immunosorbent assay. *J Immunol Methods* 1991;138:285-90.
19. Bresser P, Out TA, van Alphen L, Jansen HM, Lutter R. Airway inflammation in nonobstructive and obstructive chronic bronchitis with chronic haemophilus influenzae airway infection: comparison with noninfected patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2000;162:947-52.
20. McNamee LA, Fattah DI, Baker TJ, Bains SK, Hissey PH. Production, characterisation and use of monoclonal antibodies to human

- interleukin-5 in an enzyme-linked immunosorbent assay. *J Immunol Methods* 1991;141:81-8.
21. Nocker RE, Weller FR, Out TA, de Riemer MJ, Jansen HM, Van Der Zee JS. A double-blind study on the effect of inhaled corticosteroids on plasma protein exudation in asthma. *Am J Respir Crit Care Med* 1999;159:1499-505.
 22. Weersink EJ, Aalbers R, Koeter GH, Kauffman HF, De Monchy JG, Postma DS. Partial inhibition of the early and late asthmatic response by a single dose of salmeterol. *Am J Respir Crit Care Med* 1994;150:1262-7.
 23. Krouwels FH, Nocker RE, Snoek M, Lutter R, van der Zee JS, Weller FR, et al. Immunocytochemical and flow cytometric detection of intracellular IL-4, IL-5 and IFN-gamma: applications using blood- and airway-derived cells. *J Immunol Methods* 1997;203:89-101.
 24. Oettgen HC, Geha RS. IgE regulation and roles in asthma pathogenesis. *J Allergy Clin Immunol* 2001;107:429-40.
 25. Okayama Y, Petit-Frere C, Kassel O, Semper A, Quint D, Tunon-de-Lara MJ, et al. IgE-dependent expression of mRNA for IL-4 and IL-5 in human lung mast cells. *J Immunol* 1995;155:1796-808.
 26. Bradding P, Okayama Y, Howarth PH, Church MK, Holgate ST. Heterogeneity of human mast cells based on cytokine content. *J Immunol* 1995;155:297-307.
 27. Butchers PR, Vardey CJ, Johnson M. Salmeterol: a potent and long-acting inhibitor of inflammatory mediator release from human lung. *Br J Pharmacol* 1991;104:672-6.
 28. Bissonnette EY, Befus AD. Anti-inflammatory effect of beta 2-agonists: inhibition of TNF-alpha release from human mast cells. *J Allergy Clin Immunol* 1997;100:825-31.
 29. Gronneberg R, Lagerholm B. Histopathological support of antagonism of allergen-induced mast cell mediator release in human skin by a beta-2-agonist. *Allergy* 1986;41:71-4.
 30. Hamelmann E, Oshiba A, Schwarze J, Bradley K, Loader J, Larsen GL, et al. Allergen-specific IgE and IL-5 are essential for the development of airway hyperresponsiveness. *Am J Respir Cell Mol Biol* 1997;16:674-82.
 31. Jarjour NN, Calhoun WJ, Kelly EA, Gleich GJ, Schwartz LB, Busse WW. The immediate and late allergic response to segmental bronchopulmonary provocation in asthma. *Am J Respir Crit Care Med* 1997;155:1515-21.
 32. Van Der Veen MJ, Van Neerven RJ, De Jong EC, Aalberse RC, Jansen HM, Van Der Zee JS. The late asthmatic response is associated with baseline allergen-specific proliferative responsiveness of peripheral T lymphocytes in vitro and serum interleukin-5. *Clin Exp Allergy* 1999;29:217-27.
 33. Hamelmann E, Gelfand EW. Role of IL-5 in the development of allergen-induced airway hyperresponsiveness. *Int Arch Allergy Immunol* 1999;120:8-16.
 34. Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;356:2144-8.
 35. Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS. Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am J Respir Crit Care Med* 2003;167:199-204.
 36. Liu LY, Sedgwick JB, Bates ME, Vrtis RF, Gern JE, Kita H, et al. Decreased expression of membrane IL-5 receptor alpha on human eosinophils, I: loss of membrane IL-5 receptor alpha on airway eosinophils and increased soluble IL-5 receptor alpha in the airway after allergen challenge. *J Immunol* 2002;169:6452-8.
 37. Currie GP, Syme-Grant NJ, McFarlane LC, Carey FA, Lipworth BJ. Effects of low dose fluticasone/salmeterol combination on surrogate inflammatory markers in moderate persistent asthma. *Allergy* 2003;58:602-7.

Did you know?

You can now personalize the *JACI* Web site to meet your individual needs. Enjoy these new benefits and more:

- Search across 400 top medical and health sciences journals online, including MEDLINE.
- Greater cross-referencing results from your online searches.

Visit www.jacionline.org today to see what else is new online!