

(S,S)-Formoterol Increases the Production of IL-4 in Mast Cells and the Airways of a Murine Asthma Model

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Key Words

(R,R)-formoterol · (S,S)-formoterol · Mast cells · Interleukin-4

Abstract

Background: Racemic formoterol is an equimolar mixture of (R,R)- and (S,S)-formoterol. Several studies have shown (S,S)-formoterol to have proinflammatory effects. We previously reported that (S)-albuterol increased the secretion of histamine and interleukin (IL)-4 in murine mast cells. We thus hypothesized that (S,S)-formoterol promotes asthma by enhancing IL-4 production in mast cells of the asthmatic airway. **Methods:** Murine and human mast cells were stimulated by high affinity IgE receptor (FcεRI) cross-linking or with phorbol myristate acetate/calcium ionophore A23187 (PMA/A23187). Jurkat T cells were stimulated with PMA. Cells were pretreated with either (R,R)- or (S,S)-formoterol. Ovalbumin (OVA)-sensitized BALB/c mice were pretreated with (R,R)- or (S,S)-formoterol before each intranasal OVA challenge for 10 days. Bronchoalveolar lavage fluid was obtained from the mice. The levels of IL-4, histamine and

PGD₂ were measured. Early and late allergic responses (EAR and LAR, respectively) to OVA challenge and airway hyperresponsiveness (AHR) were measured. **Results:** (S,S)-formoterol enhanced the production of IL-4, histamine, and PGD₂ in mast cells, whereas (R,R)-formoterol had no effect. Neither (S,S)- nor (R,R)-formoterol had effect on IL-4 production in Jurkat T cells. In OVA-challenged mice, (S,S)-formoterol increased IL-4 secretion, whereas (R,R)-formoterol had no effect. Finally, (S,S)-formoterol enhanced the inflammatory changes in the peribronchial and perivascular areas without affecting EAR, LAR or AHR, whereas (R,R)-formoterol reduced EAR, LAR and AHR as well as cellular infiltration in the lung tissue of these mice. **Conclusion:** (S,S)-formoterol may exert adverse effects in asthma control by activating mast cells to produce proinflammatory mediators such as IL-4.

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Introduction

Short-acting selective β_2 -agonists (SABAs) such as albuterol are widely used to manage acute airway obstruction in patients with asthma [1]. However, the bronchodilatory action persists for less than 3–4 h [1]. Long-acting selective β_2 -agonists (LABAs) such as formoterol with a more protracted duration of action have been introduced into asthma treatment [2].

Formoterol is a racemate consisting of a 50:50 mixture of (R,R)- and (S,S)-isomers. (S,S)-formoterol has 1,000 times less receptor binding affinity to β_2 -adrenergic receptors (β_2 -ARs) than (R,R)-formoterol and very low intrinsic efficacy as evidenced by low cAMP production [3]. In isolated guinea pig tracheal strips pretreated with (S,S)-formoterol contractions in response to high concentrations of carbachol were exaggerated [3]. This study suggests an adverse effect of (S,S)-formoterol on airway relaxation.

(R,R)-formoterol reduced the levels of granulocyte macrophage-colony stimulating factor (GM-CSF), an important inflammatory cytokine, to 40% of the control value in human airway smooth muscle cells [4]. On the contrary, (S,S)-formoterol increased the production of GM-CSF [4], suggesting a proinflammatory effect of this isomer. Although studies suggest that (S,S)-formoterol may have an adverse effect on asthma control, the role of (S,S)-formoterol in mast cell mediator release is not known.

We previously reported that (S)-albuterol increased the secretion of histamine and interleukin (IL)-4 in IgE-stimulated murine mast cells [5]. Mast cells play an important role in initiating and perpetuating the inflammatory response in asthma by secreting abundant amounts of proinflammatory mediators such as histamine, prostaglandin (PG) D_2 , IL-4, IL-5, IL-6, tumor necrosis factor (TNF)- α , and leukotrienes [6]. In particular, IL-4 is an important eosinophil chemoattractant by inducing endothelial cells to produce eosinophil chemotactic factor and eotaxin [7] and by enhancing endothelial cell surface adhesion molecules such as vascular cell adhesion molecule-1 [8]. IL-4 is also essential in IgE production [9] and promotes the switch from naive T cells to the allergic type Th2 cells [10, 11]. We thus hypothesized that (S,S)-formoterol promotes IL-4 production in mast cells and in the airways of a murine asthma model, resulting in enhanced inflammatory changes and reduced lung function.

Material and Methods

Cell Culture

Cells from human mast cell line HMC-1 (a kind gift from J. H. Butterfield, Mayo Clinic, Rochester, Minn., USA) and the IL-3-independent cloned murine mast cell line C1.MC/C57.1 (a kind gift from S. Galli, Stanford University, Palo Alto, Calif., USA) were maintained as described [12, 13]. Briefly, C1.MC/C57.1 cells were cultured in Dulbecco's modified eagle medium (DMEM) (Gibco-BRL, Grand Island, N.Y., USA) supplemented with 10% heat inactivated fetal bovine serum (FBS), 50 mM β -mercaptoethanol, 4 mM *L*-glutamine, and 100 μ g/ml penicillin/streptomycin (complete DMEM) in a humidified 5% CO_2 incubator. HMC-1 cells were cultured in Iscove's modified Dulbecco's medium (Gibco-BRL) with 10% FBS, 4 mM *L*-glutamine, and antibiotics in a humidified 5% CO_2 incubator. Jurkat T cells were obtained from American Type Culture Collection (ATCC, Rockville, Md., USA) and grown in complete RPMI 1640 medium supplemented with 10% FCS. The cell number was adjusted to 5×10^5 cells/ml twice weekly by adding fresh media.

Stimulation Conditions

For Fc ϵ RI-dependent activation, C1.MC/C57.1 cells were adjusted to a density of 0.5×10^6 cells/ml in complete DMEM, 0.5 μ g/ml of IgE anti-DNP added, and the cells incubated at 37°C for 60 min in humidified 5% $CO_2/95\%$ air with gentle rocking. Cells were then centrifuged at 400 *g* at 4°C for 10 min and the cell pellets were resuspended in fresh media. The resuspended cells were treated with 10^{-7} M (R,R)- or (S,S)-formoterol 10 min before antigen stimulation. To assess dose response, varying concentrations ranging from 10^{-9} to 10^{-6} M of (S,S)-formoterol were used. Antigen-mediated degranulation was performed by addition of DNP_{30–40}-human serum albumin (Sigma-Aldrich, St. Louis, Mo., USA) to a final concentration of 40 ng/ml followed by continued incubation with gentle rocking at 37°C. Following incubation with antigen in the presence of varying concentrations of either (R,R)- or (S,S)-formoterol or no drug, the cells were then centrifuged (10 min, 200 *g* at 4°C) and the cell-free supernatants from each group were collected for IL-4 assay. HMC-1 cells were stimulated with PMA (Sigma-Aldrich) 30 ng/ml and A23187 (Sigma-Aldrich) 350 ng/ml and Jurkat T cells were stimulated with PMA (50 ng/ml) in the presence of varying concentrations of either (R,R)- or (S,S)-formoterol, or no drug. The cells were centrifuged (10 min, 200 *g* at 4°C) and samples of the cell-free supernatant from each group were collected for ELISA.

Animals and Induction of Murine Asthma

Six- to 8-week old, male BALB/c mice were obtained from the Jackson Laboratory (Bar Harbor, Me., USA). All experimental procedures complied with the requirements of the Animal Care and Use Committee of the Harbor-UCLA Research Education Institute. Mice were housed in autoclaved cages on autoclaved bedding in an air-conditioned room on a 12-hour light/dark cycle. The mice were sensitized with intraperitoneal injection of 1 mg of alum precipitated chicken egg ovalbumin (OVA; grade V, 98% pure, Sigma) in 0.1 ml phosphate-buffered saline (PBS) at 7 and 14 days prior to nasal OVA challenge. The animals were divided into four groups and lightly anesthetized by inhalation of methoxyflurane (Metofane) and subjected to a single intranasal challenge with 100 μ l of 1% OVA or PBS daily for 10 days. The mice were pretreated with either 0.5 μ g of (R,R)- or (S,S)-formoterol 1 h before OVA or PBS challenge. Twenty-four hours after the last OVA challenge on day 10, pulmonary func-

tion was monitored before (baseline) and after single OVA-inhalation challenge for 30 min to examine EAR of asthma using Buxco BioSystem Plethysmograph (Buxco XA, Buxco, Troy, N.Y., USA). In order to investigate LAR of asthma, pulmonary function was recorded every hour after 1 through 9 h following OVA-inhalation challenge. The isomers were again administered 2–4 h before the AHR measurement, which was examined at 24 h after OVA-inhalation challenge by measuring the airway resistance to methacholine. At the end of the experiment the mice were sacrificed. The trachea was cannulated and bronchoalveolar lavage fluid (BALF) was obtained for IL-4 assay.

Measurement of IL-4, Histamine and PGD₂

The presence of IL-4 in the cell culture supernatants and BALF was determined in duplicate by ELISA (Endogen, Woburn, Mass., USA) according to the manufacturer's protocol. The detection limits of the ELISA kits were 3–5 pg/ml. This ELISA did not cross-react with other cytokines including human or mouse IL-3, IL-5, IL-6, IL-10, interferon (IFN)- γ , GM-CSF, and TNF- α . The levels of PGD₂ (Cayman Chemical, Ann Arbor, Mich., USA) and histamine (Beckman Culture, Inc., Fullerton, Calif., USA) were measured by ELISA according to the manufacturer's protocol. The minimal quantities of released histamine and PGD₂ detected in the resting samples were subtracted from the other samples in each experiment, and the data were expressed as the net production in response to each stimulant based on the mean of the replicate experiments indicated in the text.

Histopathologic Analysis of Lung Tissue

Mice were sacrificed 24 h after their last OVA or PBS inhalation exposure. Lungs were removed from the chest cavity and inflated with 1 ml neutral buffered 10% formalin at a fixed pressure of 25 cm and fixed overnight in 10% formalin. The lungs were then embedded in paraffin. Sections were stained with hematoxylin and eosin for examining inflammatory changes. The cells in the peribronchial area were counted as a number of inflammatory cells/mm² as described [14, 15]. Five to seven peribronchial infiltrates per animal were scored.

Methacholine Challenge

To measure methacholine responsiveness, mice were exposed for 2 min to PBS, followed by incremental dosages (3–50 mg/ml) of aerosolized methacholine (freshly prepared in PBS); Penh was monitored for each dosage. Results were expressed for PBS and methacholine as the percentage of baseline Penh values before PBS exposure. Pulmonary function was monitored using the Buxco BioSystem Plethysmograph with a flow-derived analyzer as described previously in our paper [16]. AHR was expressed as an index of airway reactivity in P_{enh} units, which reflect 'Effort of Breathing'. The Buxco BioSystem is ideally suited for long-term measurements in conscious animals.

Statistics

Differences in IL-4 concentrations in ELISA assays were determined by the Student's unpaired sample t test (two-tailed). Mann-Whitney U test for comparison was used to compare difference between means obtained in EAR and LAR assessments. Analysis of variance was conducted for multiple comparisons. Significance was set at p values <0.05.

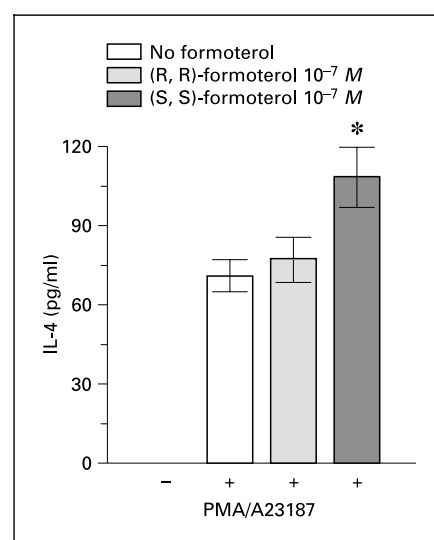


Fig. 1. IL-4 secretion from HMC-1 cells either unstimulated or stimulated with PMA/A23187 overnight. Mast cells were treated with either (R,R)- or (S,S)-formoterol 10 min before PMA/A23187 at a concentration of 10⁻⁷ M. Untreated mast cells were used as controls. Cell-free supernatants from each group were measured in duplicate by ELISA (Endogen) according to the manufacturer's protocol. Data presented are mean ± SEM of five independent experiments performed in duplicate; * p < 0.05 compared with the formoterol untreated group.

Results

IL-4 Secretion by Human Mast Cells

To determine whether (S,S)-formoterol enhances IL-4 production in human mast cells, we measured IL-4 protein concentrations by ELISA in HMC-1 cells after stimulation with PMA/A23187. After exposure to (S,S)-formoterol 10⁻⁷ M, IL-4 secretion was augmented by 51% relative to untreated stimulate cells (107.8 ± 11.5 vs. 71.5 ± 5.3 pg/ml; n = 5, p < 0.05) (fig. 1). (R,R)-formoterol 10⁻⁷ M had no significant effect on IL-4 production (77.8 ± 7.8 vs. 71.5 ± 5.3 pg/ml; n = 5, p > 0.05).

IL-4 Secretion by Murine Mast Cells

To determine whether (S,S)-formoterol 10⁻⁷ M enhances IL-4 production in murine mast cells, we stimulated cells with DNP to allow FcεRI cross-linking, a major physiologic stimulation for mast cells, and measured IL-4 concentrations by ELISA. IL-4 secretion was increased in FcεRI-stimulated mast cells by 70% upon exposure to (S,S)-formoterol relative to untreated stimulate cells (44.7 ± 5.8 vs. 26.3 ± 3.0 pg/ml; n = 5, p < 0.05) (fig. 2A).

Fig. 2. IL-4 secretion from murine mast cells either unstimulated or stimulated by FcεRI cross-linking overnight. **A** Mast cells were treated with either (R,R)- or (S,S)-formoterol at a concentration of 10^{-7} M 10 min before FcεRI cross-linking. **B** Mast cells were treated with (S,S)-formoterol at concentrations from 10^{-9} to 10^{-6} M 10 min before FcεRI cross-linking. Untreated mast cells were used as controls. Cell-free supernatants from each group were measured in duplicate by ELISA (Endogen) according to the manufacturer's protocol. Data presented are mean $\pm$ SEM of five independent experiments performed in duplicate; * $p < 0.05$ and ** $p < 0.001$ compared with the formoterol untreated group.

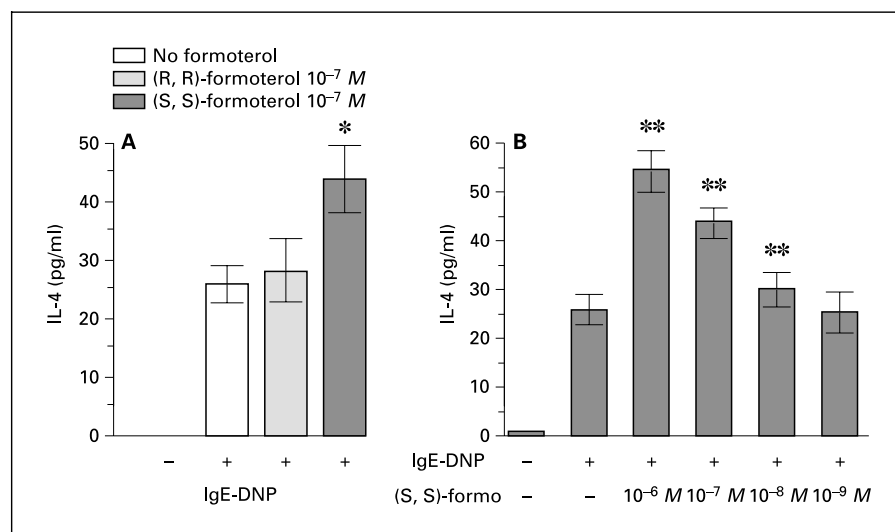
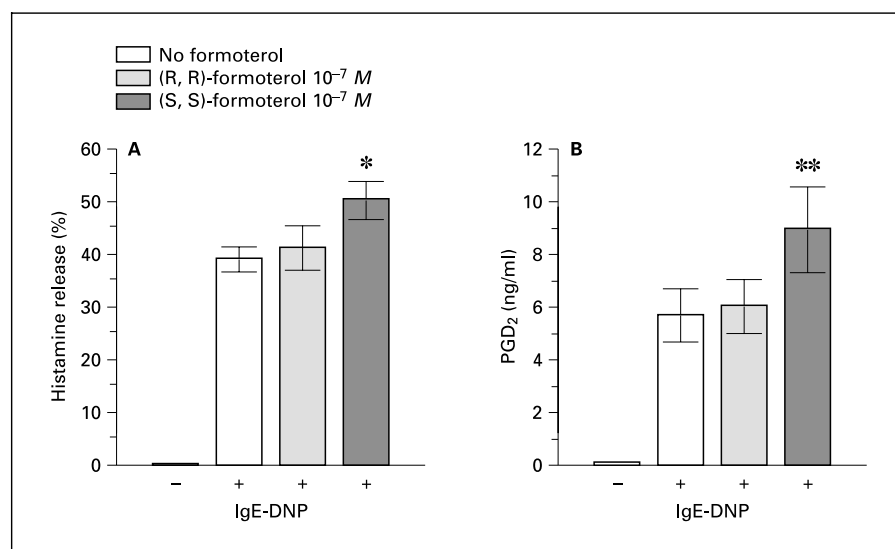


Fig. 3. **A** Histamine release from murine mast cells either unstimulated or stimulated by FcεRI cross-linking for 30 min. **B** PGD₂ secretion from murine mast cells either unstimulated or stimulated by FcεRI cross-linking for 30 min. Mast cells were treated with either (R,R)- or (S,S)-formoterol at a concentration of 10^{-7} M 10 min before FcεRI cross-linking at a concentrations of 10^{-7} M. Untreated mast cells were used as controls. Cell-free supernatants from each group were analyzed for histamine or PGD₂ in duplicate by ELISA according to the manufacturer's protocol. Data presented are mean $\pm$ SEM of four independent experiments performed in duplicate; * $p < 0.005$ and ** $p < 0.02$ compared with the formoterol untreated group.



In the (R,R)-formoterol 10^{-7} M treated cells, however, IL-4 concentrations were not significantly changed relative to untreated stimulated cells (28.7 ± 5.5 vs. 26.3 ± 3.0 pg/ml; $n = 5$, $p > 0.05$). To further clarify the effect of (S,S)-formoterol on IL-4 production in mast cells, a dose-response study was performed. In (S,S)-formoterol-treated stimulate cells, IL-4 production was enhanced in a dose-dependent manner compared with untreated stimulate cells ($n = 5$, $p < 0.001$) (fig. 2B).

Secretion of Histamine and PGD₂ by Mast Cells

To determine whether (S,S)-formoterol also enhances the secretion of histamine and PGD₂ by mast cells, we stimulated murine mast cells with DNP to allow FcεRI

cross-linking and measured histamine and PGD₂ concentrations by ELISA. In IgE-stimulated mast cells, histamine release was increased by 13% upon exposure to (S,S)-formoterol 10^{-7} M relative to untreated IgE-stimulated cells (51.2 ± 3.4 vs. $39.4 \pm 2.3\%$; $n = 4$, $p < 0.005$) (fig. 3A). In the (R,R)-formoterol 10^{-7} M treated IgE-stimulated cells, however, histamine release was not significantly changed (41.9 ± 3.9 vs. $39.4 \pm 2.3\%$; $n = 4$, $p > 0.05$). PGD₂ secretion was also increased by 57% upon exposure to (S,S)-formoterol relative to untreated IgE-stimulated cells (9.1 ± 1.7 vs. 5.8 ± 1.0 ng/ml; $n = 4$, $p < 0.02$) (fig. 3B). In contrast, (R,R)-formoterol did not enhance PGD₂ production (6.1 ± 1.0 vs. 5.8 ± 1.0 ng/ml; $n = 4$, $p > 0.05$).

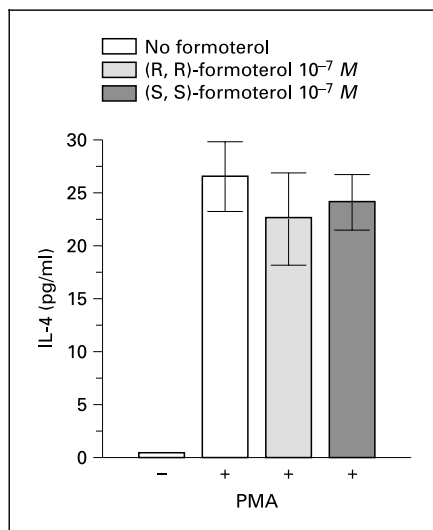


Fig. 4. IL-4 secretion from Jurkat T cells either unstimulated or stimulated with PMA overnight. T cells were treated with either (R,R)- or (S,S)-formoterol at a concentration of 10^{-7} M 10 min before PMA. Untreated mast cells were used as controls. Cell-free supernatants from each group were measured in duplicate by ELISA (Endogen) according to the manufacturer's protocol. Data presented are mean $\pm$ SEM of four independent experiments performed in duplicate. No significant differences noted.

IL-4 Secretion in Jurkat T Cells

To determine whether (S,S)-formoterol enhances IL-4 secretion in other cells such as T lymphocytes, we measured IL-4 protein concentrations by ELISA in Jurkat T cells after stimulation with PMA. Neither (S,S)- nor (R,R)-formoterol 10^{-7} M treatment significantly changed IL-4 secretion by Jurkat T cells compared with untreated Jurkat T cells (24.3 ± 2.6 vs. 26.8 ± 3.3 pg/ml or 22.8 ± 4.2 vs. 26.8 ± 3.3 pg/ml; $n = 4$, $p > 0.05$) (fig. 4).

IL-4 Secretion in the Airway of a Murine Asthma Model

To evaluate whether (S,S)-formoterol enhances IL-4 secretion in the airways of a murine asthma model, mice were sensitized with intraperitoneal OVA and challenged with intranasal OVA. IL-4 concentration in the BALF from mice treated with (S,S)-formoterol 0.5 μ g/day for 10 days was increased by 82% compared with that of mice not treated with formoterol (98.3 ± 10.8 vs. 54.0 ± 7.3 pg/ml; $n = 5$, $p < 0.02$) (fig. 5). In the BALF from mice treated with (R,R)-formoterol 0.5 μ g/day for 10 days, IL-4 concentration was not significantly changed relative to those that received no formoterol (57.3 ± 9.0 vs. 54.0 ± 7.3 pg/ml; $n = 5$, $p > 0.05$).

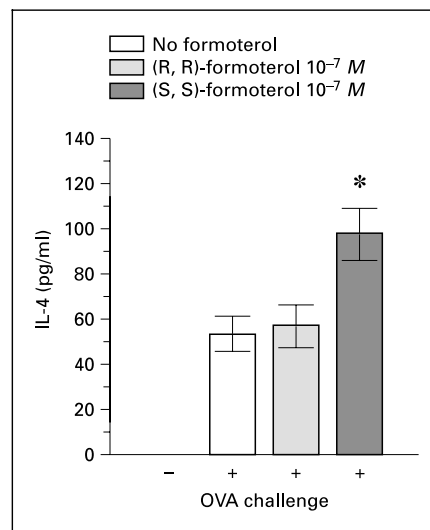


Fig. 5. IL-4 secretion in BALF from murine airways after challenge with OVA or PBS. Mice were pretreated with either 0.5 μ g of (R,R)- or (S,S)-formoterol daily for 10 days. BALF from each group was obtained for IL-4 assay and was measured in duplicate by ELISA (Endogen) according to the manufacturer's protocol. Data presented are mean $\pm$ SEM of five independent experiments performed in duplicate; * $p < 0.02$ compared with the formoterol untreated group.

Histopathology of the Lungs

To examine whether (S,S)-formoterol enhances inflammatory changes in the airways of a murine asthma model, histopathological changes in lung tissue from mice were evaluated after OVA or PBS challenge. In the PBS-treated control mice, inflammatory cells were absent in the peribronchial and perivascular areas (fig. 6A). However, after OVA challenge, dense inflammatory cell infiltrates, consisting of eosinophils and mononuclear cells, were observed in these areas (fig. 6B). (S,S)-formoterol 0.5 μ g treatment further enhanced the cellular infiltration in these areas (fig. 6C), whereas (R,R)-formoterol 0.5 μ g treatment reduced the cellular infiltration in these areas relative to OVA-treated mice not exposed to formoterol (fig. 6D). In quantitation of peribronchial infiltrates, the numbers of both of eosinophils and mononuclear cells were significantly higher in mice pretreated with (S,S)-formoterol compared with those not treated with formoterol ($n = 5$, $p < 0.005$ and $p < 0.01$, respectively) (fig. 7A, B). On the other hand, the number of peribronchial eosinophils and mononuclear cells were significantly lower in mice treated with (R,R)-formoterol compared with those not treated ($n = 5$, $p < 0.001$ and $p < 0.005$, respectively).

Fig. 6. Pathologic changes of OVA-challenged mice. **A** In the PBS-treated control mice, inflammatory cells are absent in the lung interstitium around airways (AW) and blood vessels (BV). **B** In the OVA-treated mice, a dense inflammatory cell infiltrate around the airways (AW) and blood vessels (BV) is observed. Eosinophils and mononuclear cells are the predominant cells in this infiltrate. **C** (S,S)-formoterol enhanced the cellular infiltration of the lung interstitium around the airways (AW). **D** (R,R)-formoterol reduced the cellular infiltration of the lung interstitium around the airways (AW). M denotes mucus plug inside airway. The photomicrographs were selected to illustrate the pattern and extent of inflammatory changes for each slide. **A–D** Magnification: $\times 400$.

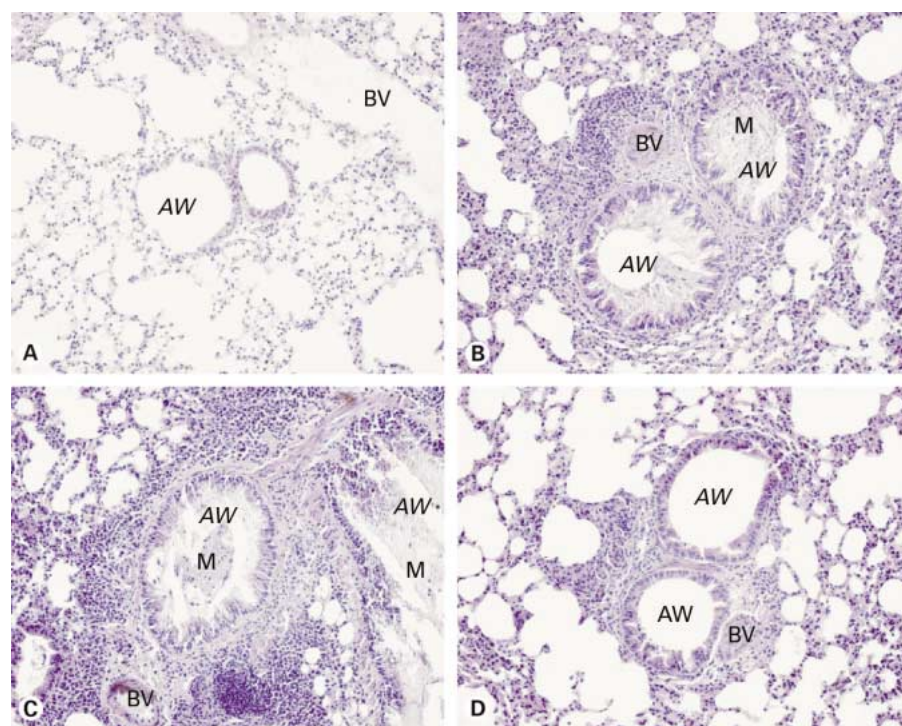
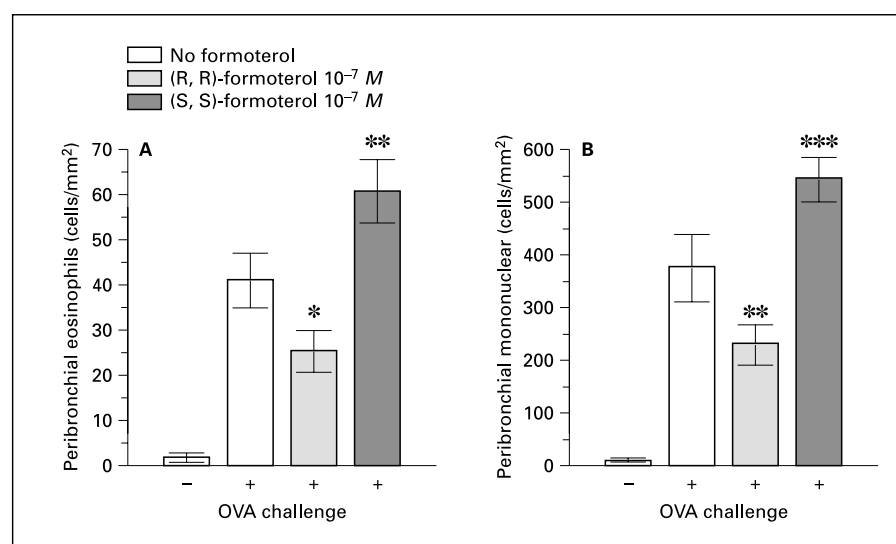


Fig. 7. Cellular composition of peribronchial infiltration. **A** Peribronchial infiltration of eosinophils. **B** Peribronchial infiltration of mononuclear cells. Data presented are mean $\pm$ SEM of five independent experiments performed in five to seven areas of peribronchial infiltration per animal. * $p < 0.001$, ** $p < 0.005$ and *** $p < 0.01$ compared with the formoterol untreated group.

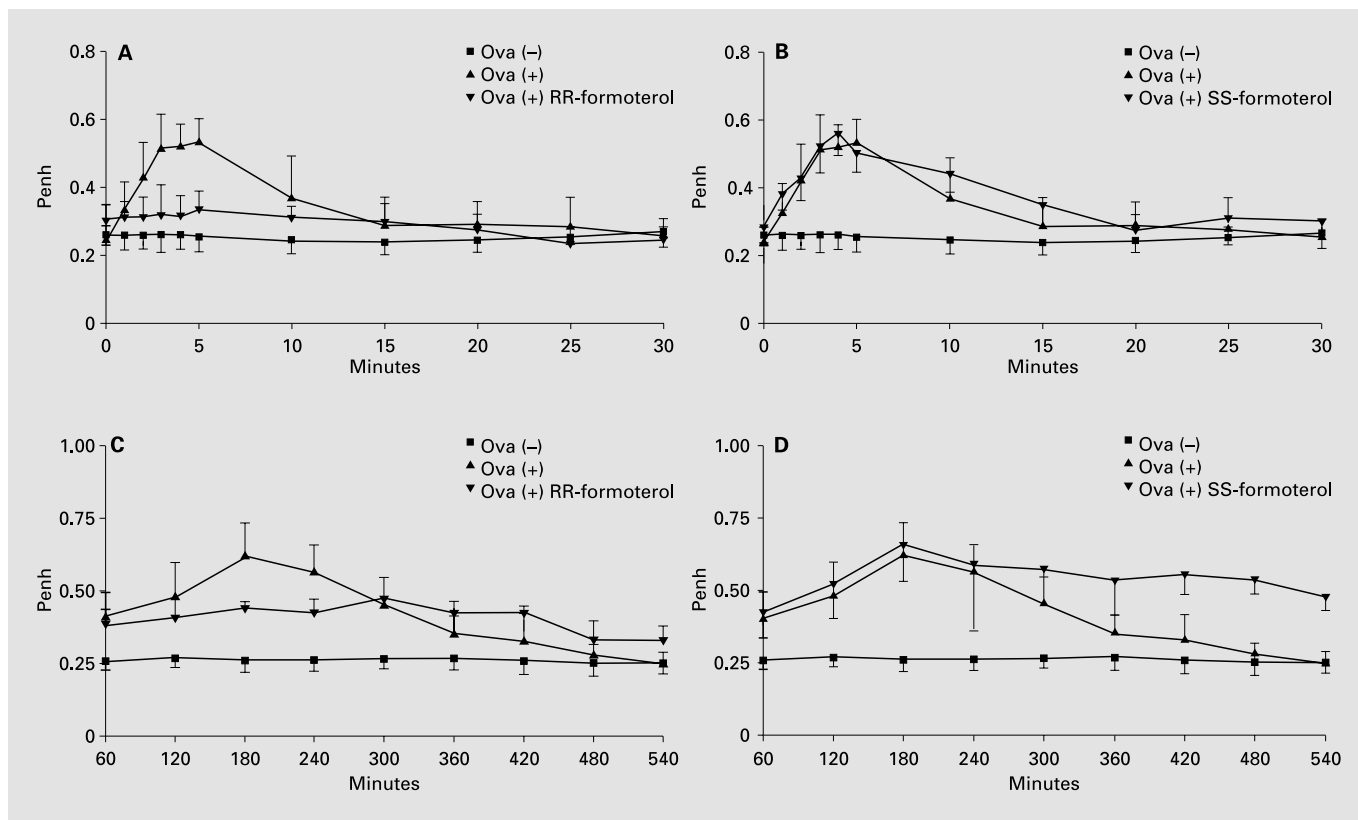


Effect of Formoterol Stereoisomers on Early and Late Allergic Response and Airway Hyperresponsiveness

To examine whether (S,S)-formoterol enhances airway reactivity in a murine asthma model, airway physiological studies were performed after OVA or PBS challenge. There was a significant reduction in the early allergic response by (R,R)-formoterol 0.5 μ g/day for 10 days (fig. 8A), whereas (S,S)-formoterol 0.5 μ g/day for 10 days had no effect on EAR (fig. 8B). (R,R)-formoterol signifi-

cantly suppressed the LAR (fig. 8C), whereas (S,S)-formoterol showed no effect on LAR (fig. 8D). Interestingly, however, (S,S)-formoterol increased the basal tone of the airways (fig. 8D).

AHR to methacholine was measured 24 h after the final OVA challenge. (R,R)-formoterol significantly attenuated AHR to methacholine (fig. 9A), whereas (S,S)-formoterol showed no effect on AHR to methacholine (fig. 9B).



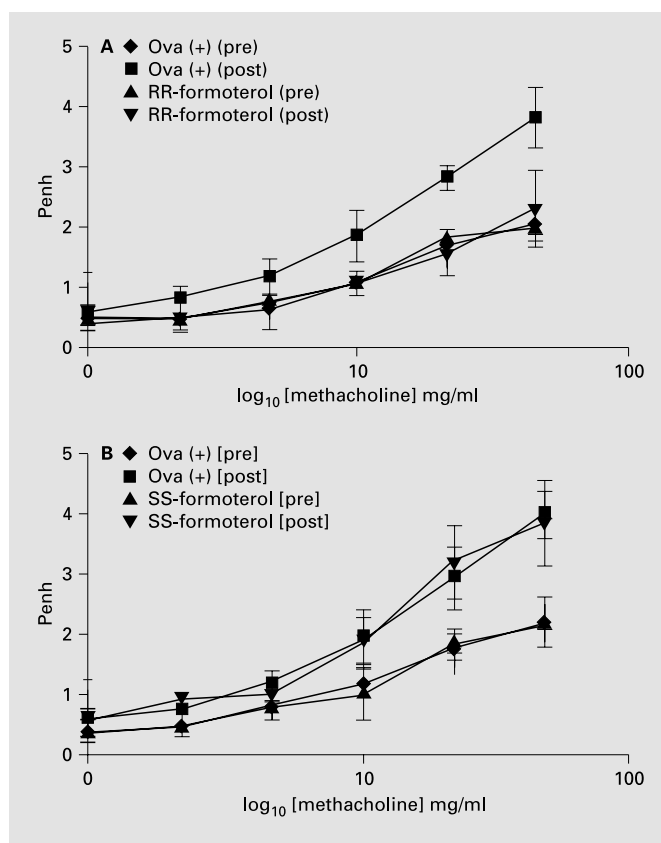
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Fig. 8. Effect of treatment with formoterol 0.5 $\mu\text{g/day}$ for 10 days on EAR and LAR after challenge with OVA or PBS. **A** Effect of (R,R)-formoterol on EAR. **B** Effect of (S,S)-formoterol on EAR. **C** Effect of (R,R)-formoterol on LAR. **D** Effect of (S,S)-formoterol on LAR. P_{enh} values were recorded in individual animals. The data are representative of 6 animals in each experimental group.

Fig. 9. Effect of formoterol on airway hyperresponsiveness to methacholine after challenge with OVA or PBS. **A** Effect of (R,R)-formoterol on airway hyperresponsiveness. **B** Effect of (S,S)-formoterol on airway hyperresponsiveness. P_{enh} values were recorded in individual animals. The data are representative of 6 animals in each experimental group.

Discussion

In this study, we demonstrated that (S,S)-formoterol significantly increased IL-4 secretion in a dose-dependent manner by stimulated human and murine mast cells, whereas (R,R)-formoterol had no significant effect on IL-4 secretion in either human or murine mast cells. IL-4 protein was not detected in unstimulated murine or human mast cells. Other studies also reported that IL-4 protein was not detected in the supernatants of unstimulated mast cells, although transient IL-4 mRNA expres-



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sion was found [17, 18]. Formoterol is a racemate consisting of a 50:50 mixture of (R,R)- and (S,S)-isomers. The (R,R)-isomer of formoterol has 1,000 times greater binding affinity to β_2 -ARs than the (S,S)-isomer. In addition, (S,S)-formoterol has very low intrinsic efficacy as evidenced by low cAMP production [3]. Functional β_2 -ARs have been detected in lung mast cells, and both SABAs and LABAs inhibit inflammatory mediator release from these cells [19]. Salmeterol also has been reported to inhibit IgE-dependent release of TNF- α from human mast cells [20]. However, the effect of individual isomers of formoterol on mast cell mediator release had not been investigated. The previous finding that it is the (R,R)-isomer of formoterol that binds more tightly to and exerts its action via β_2 -ARs and our finding that (R,R)-formoterol had no effect on IL-4 secretion by mast cells suggest that mast cell IL-4 secretion may occur independently of β_2 -ARs. This hypothesis is further supported by our data demonstrating that (S,S)-formoterol, which exerts its effect via essentially a non- β_2 -AR-mediated mechanism, increases IL-4 secretion, although further studies are required to determine whether blocking the β_2 -AR with a β_2 -AR blocker such as propranolol inhibits (S,S)-formoterol-mediated IL-4 secretion.

In this study, (S,S)-formoterol also amplified secretion of histamine and PGD₂ by IgE-stimulated murine mast cells. These data are consistent with our previous report that (S)-albuterol increases histamine release in IgE-stimulated mast cells [5]. However, the effect of (S,S)- or (R,R)-formoterol on mast cell histamine release may vary depending on species. For example, Lau et al. [21] reported that racemic formoterol inhibits histamine release in isolated lung mast cells of guinea pig and human but not in rat peritoneal mast cells.

T lymphocytes are one of the most important sources of IL-4 in the pathogenesis of asthma. In our study, (S,S)-formoterol had no effect on IL-4 secretion in Jurkat human T cells in contrast to its stimulatory effect on IL-4 production in mast cells, suggesting that the effect of (S,S)-formoterol on IL-4 production is cell type-specific. Our results are consistent with Choy et al.'s finding [22] that albuterol did not have any effect on the expression of the IL-4 gene in human peripheral blood CD4⁺ T cells.

We also demonstrated that (S,S)-formoterol increased IL-4 secretion in the airways of a murine asthma model, whereas (R,R)-formoterol had no significant effect on IL-4 secretion in these mice. Whether mast cells or other cell types such as T cells and eosinophils are a major source of IL-4 in the airways of these mice remains to be determined [23], although our *in vitro* data demonstrated

that IL-4 production is significantly enhanced in IgE-stimulated mast cells after exposure to (S,S)-formoterol. For example, Bradding et al. [6] reported that the majority of IL-4 protein-positive cells in bronchial tissues are mast cells, whereas Ying et al. [24] demonstrated that greater than 70% of IL-4 mRNA-positive cells in bronchial tissues are T cells.

In our histopathologic study, mice treated with (S,S)-formoterol showed enhanced peribronchial and perivascular inflammation, whereas those treated with (R,R)-formoterol showed reduced cellular infiltration compared with untreated mice. In further quantitative analysis of individual infiltrating cells, the numbers of mononuclear cells and eosinophils in mice pretreated with (R,R)-formoterol were significantly lower than those without formoterol treatment. These data suggest that (R,R)-formoterol has an anti-inflammatory effect as well as a bronchodilator effect. These findings are consistent with those of other reports demonstrating the anti-inflammatory effects of formoterol [25–27]. For example, regular treatment with racemic formoterol reduced the number of inflammatory cells in the mucosal layer and the number of submucosal mast cells in the human asthmatic airway [25]. Racemic and (R,R)-formoterol reduced the number of total inflammatory cells and eosinophils in BALF in a dose-dependent manner [25]. In this study, (R,R)-formoterol effectively inhibited cellular infiltration, whereas (S,S)-formoterol enhanced the infiltration. These data suggest that (S,S)-formoterol may attenuate the anti-inflammatory effect of (R,R)-formoterol in the asthmatic airway. The evidence of (R,R)-formoterol's anti-inflammatory effect is further supported by demonstrating that (R,R)-formoterol also reduced levels of GM-CSF, an important inflammatory cytokine, in human airway smooth muscle cells [4]. However, the mechanism by which (R,R)-formoterol may exert its anti-inflammatory effect in the lung of a murine asthma model remains elusive, although our data suggest that the effect is not by regulating an IL-4-dependent mechanism.

(R,R)-formoterol significantly reduced the EAR and LAR. Furthermore, (R,R)-formoterol significantly attenuated the AHR to methacholine. On the other hand, (S,S)-formoterol has no effect on EAR, LAR, and AHR. Interestingly however, there was a prolonged increase in the basal tone of the airways after treatment with (S,S)-formoterol in experiments examining the EAR and LAR. These data suggest that (S,S)-formoterol has no effect on bronchodilatory effect but it might affect airway patency.

In summary, (S,S)-formoterol may have adverse effects in asthma control at least through increasing the secretion

of mast cell mediators such as IL-4, histamine and PGD₂. Moreover, the mechanism by which (S,S)-formoterol exerts its effect on IL-4 production appears to be a non- β_2 -AR-mediated mechanism, since (R,R)-formoterol, which exerts its action via β_2 -ARs, had no effect on IL-4 secretion, whereas (S,S)-formoterol significantly enhanced IL-4 secretion. Experiments are underway to confirm this hypothesis by determining whether blocking the β_2 -AR with propranolol inhibits (S,S)-formoterol-mediated IL-4 secretion. Further studies are necessary to determine the mechanism by which (S,S)-formoterol exerts its

adverse effects on mast cell mediator release and inflammatory changes in the airways of a murine model of asthma. This could have implications for treating patients with asthma.

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