Effects of LABA on the production of Th1- and Th2- related chemokines by monocytes and bronchial epithelial cells

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Running title: Effects of formoterol and salmeterol on chemokines

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Key words: bronchial epithelial cells, bronchodilators, CCL chemokines, monocyte/macrophage, β2 agonist

This study was supported by grants from National Science Council (95-2314-B-037-092) of the Republic of China.
Abstract

The question of the study: It is not known whether formoterol and salmeterol, two long-acting $\beta_2$-adrenoreceptor agonists (LABAs), have regulatory functions in Th2- and Th1- related chemokines in monocytes and bronchial epithelial cells.

Material and Methods: We investigated the effects of two LABAs on lipopolysaccharide (LPS)-induced expression of Th2-related chemokines, MDC/CCL22, and Th1-related chemokines, IP-10/CXCL10, in a monocytic cell line, THP-1, and human primary monocytes; also, their effects on Th2-related chemokines, TARC/CCL17 expression, in an epithelial cell line, BEAS-2B, was evaluated.

Results: Formoterol enhanced MDC, but suppressed IP-10, production in monocytes induced by LPS. Higher doses of salmeterol were required to enhance LPS-induced MDC expression in THP-1 cells. Formoterol and salmeterol could significantly suppress TARC expression in BEAS-2B cells. These effects could be reversed by a beta2-adrenoreceptor-selective antagonist, ICI-118551. Formoterol- and LPS-induced MDC expression was inhibited by budesonide.

The answer to the question: Both LABAs suppressed TARC expression in bronchial epithelial cells mediated via beta2-adrenoreceptors. Formoterol at physiologic concentration could suppress LPS-induced Th1-related (IP-10) but enhance Th2-related (MDC) chemokine expression in human monocytes. LABAs may increase Th2-related chemokine
expression in monocytes and the Th2 cell recruitment, and, therefore, LABA monotherapy may not be an appropriate therapeutic option for asthma.
**Introduction:**

Asthma is a chronic inflammatory disorder of the airway (1). Infiltration of the airways by T helper type 2 (Th2) lymphocytes, eosinophils and other inflammatory cells is a well-recognized feature of bronchial asthma (2,3). The role of Th1-type responses in asthma has been of great interest, as there have been several proposed therapies or preventive measures attempting to reduce allergic airway inflammation by enhancing the Th1 inflammatory responses (2,3). However, Th1 responses do not always downregulate allergic inflammation. IFNγ-inducible protein 10 (IP-10; CXCL10) is a chemokine that attracts Th1 lymphocytes through its receptor CXCR3 (4). IP-10 is induced in a variety of cells in response to IFNγ (4) and is also up-regulated in allergic pulmonary inflammation (5). IP-10 is expressed in airway smooth muscle of subjects with asthma when compared with those from healthy control subjects (6), suggesting that the CXCL10/CXCR3 axis may serve as a novel target for the treatment of asthma (6). Macrophage-derived chemokine (MDC)/CCL22 and thymus- and activation-regulated chemokine (TARC)/CCL17 are Th2 chemokines involved in the recruitment of CC chemokine receptor (CCR) 4-bearing Th2 cells in allergen-induced inflammation (7). Increased level of plasma MDC and TARC has been found in children with acute asthma, but its level decreases after ketotifen treatment (8).

β2-adrenoreceptor agonists are first-line drugs for the treatment of acute asthma, because of their potent and rapid bronchodilatory effects. Inhaled long-acting β2-agonists (LABAs)
are generally combined with corticosteroids as a controller of asthma and considered to be smooth muscle relaxants. While their anti-inflammatory properties are still a matter of debate, their anti-inflammatory effects are suggested by an inhibitory effect on granulocyte adhesion to epithelium (9) and on infiltration of inflammatory cells in the skin and lungs of guinea pigs (10). LABAs have also been shown to exert their inhibitory effects on the expression of pro-inflammatory cytokines (IL-6, IL-8 and TNFα) in a variety of cell types. For example, formoterol is shown to be able to suppress LPS-induced IL-6 expression in a mouse model, but enhance the expression of IL-8 in bronchial epithelial cells (11,12). Also, salmeterol has been shown to inhibit TNFα secretion in LPS-activated THP-1 cells (13) and suppress the IgE-dependent release of TNFα from human skin mast cells (14). Wallin and colleagues have recently reported the inhibition of eosinophil infiltration by formoterol in subjects with asthma (15). Also, inhaled LABA and corticosteroid give optimal control of asthma in most patients, and two fixed combination inhalers (salmeterol/fluticasone and formoterol/budesonide) have increasingly been used as a convenient controller in patients with persistent asthma. Thus, there is a strong scientific rationale for the combination of these two drug classes (16).

In this study, we investigated whether formoterol and salmeterol have regulatory effects on the expression of Th2- (MDC and TARC) and Th1- (interferon-inducible protein 10
(IP-10)/CXCL10) related chemokines in human monocytes (THP-1 cells and peripheral blood monocytes) and in bronchial epithelial cells (BEAS-2B).
Methods:

Cell preparation:

The human monocytic cell line, THP-1 (American Type Culture Collection, Rockville, MD), was cultured in RPMI 1640 medium (Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal bovine serum, 100 U/mL of penicillin, and 100 µg/mL of streptomycin at 37°C with 5% CO₂ in a humidified incubator. THP-1 cells were incubated at 37°C with 3.2 x 10⁻⁷ M PMA (SIGMA Chemical Co.; St Louis, MO). After incubating with PMA for 24 hours, THP-1 cells were differentiated into macrophage-like cells. Cells were centrifuged and resuspended in fresh media in 24-well plates at a concentration of 10⁶/mL for 24 h before experimental use. The cells were pre-treated with formoterol (10⁻¹⁰ M-10⁻⁷ M), salmeterol (10⁻¹⁰ M-10⁻⁵ M) or etazolate (10⁻⁶ M-10⁻⁵ M) 2 hours before LPS (0.2 µg/ml) or TNFα (20 ng/ml) stimulation. BEAS-2B cells were pre-treated with formoterol, salmeterol or etazolate for 2 hours before TNFα (50 ng/ml), IL-4 (50 ng/ml) and IFNγ (10 ng/ml) stimulation (17). Cell supernatants were collected 12, 24 and 48 hours after stimulation. In some cases, the cells were pre-treated with a selective β2 adrenoreceptor antagonist, ICI118551, BAY 11-7085 (Calbiochem, Cambridge, MA), fluticasone or budesonide for 1 hour before treatment of the cells with formoterol or salmeterol.
Peripheral blood samples were obtained from healthy individuals who had no personal or family history of allergic diseases (n = 3). Cord blood was harvested from the delivered placenta into a standard blood collection bag with citrate phosphate dextrose anticoagulant. We collected the cord blood from the delivered placenta to avoid any risk to the mother or infant from the collection process. The study protocol was approved by the Institution Review Board of Kaohsiung Medical University Hospital. Informed consent was obtained from three healthy adult volunteers and each parent of cord blood donors. Blood samples were diluted with an equal volume of phosphate-buffered saline. Peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient centrifugation (Lymphoprep, Oslo, Norway), and monocytes were isolated by magnetic bead sorting with anti-CD14 monoclonal antibody (mAb) (MACS, Miltenyi Biotec, Germany).

**In vitro polarization of human Th1 and Th2 cells from cord blood mononuclear cells**

Human mononuclear cells were isolated from the cord blood of healthy neonates using Ficoll Isolation Paque (Amersham Pharmacia Biotech, Uppsala, Sweden). The CD4+ lymphocytes were further purified by magnetic bead sorting with anti-CD4 monoclonal antibody (mAb) (MACS, Miltenyi Biotec, Germany). The cells were polarized as previously described (18). Th2 cultures were supplemented with 10 ug/ml of anti-IL-12 (R&D Systems, Minneapolis,
MN) and 10 ng/ml of IL-4 (R&D System, Minneapolis, MN). T cells were cultured with 5 ng/ml IL-12 and 10 ug/ml anti-IL-4 for Th1 polarization (R&D System, Minneapolis, MN). After 48 h of priming, IL-2 (R&D Systems, Minneapolis, MN) was added to the cultures. The cells were cultured in the presence of IL-2 alone without the addition of any polarizing cytokines. After 7 days of polarization, the IFN$\gamma$ and IL-4 cytokine production (data not shown) was determined with the use of ELISA (R & D system, Minneapolis, MN).

**Chemotactic assay**

Chemotaxis of Th1 and Th2 cells was measured by using a 24-well Micro Chemotaxis Transwell (Corning Costar, Cambridge, MA). The Th1 and Th2 cells were resuspended at 3 $\times$ 10$^5$/mL and loaded onto the upper chamber of the Micro Chemotaxis chamber. The supernatants of LPS-treated THP-1 cells or IL-4-, TNF$\alpha$- and IFN$\gamma$-treated BEAS-2B cells were added to the lower chamber. In some cases, both kinds of supernatants were mixed with the ratio of 1:1. The lower and upper chambers were separated by a polycarbonate membrane (5-$\mu$m pore size). The Th1 and Th2 cells were left to transmigrate for 3h at 37°C in a humidified atmosphere with 5% CO$_2$. After incubation for 3h, the number of migrated Th2 cells in the lower compartment was determined by counting the cells under light microscopy. The percentage of inhibition is calculated from three separate experiments.
**Western blotting**

After treatment for 2 h with or without salmeterol (10^-8 - 10^-6 M), the cells were stimulated with LPS (0.2 µg/ml) and lysed with equal volumes of ice-cold 150-µl lysis buffer 1 h later. After centrifugation at 13,000 × g for 15 min, equal amounts of cell lysates from each experimental condition were analyzed by Western blot with anti-IκBα and β-actin antibodies (Santa Cruz Biotechnology, Santa Cruz, CA). Immunoreactive bands were visualized using horseradish peroxidase-conjugated secondary antibody and the enhanced chemiluminescence (ECL) system (Amersham Pharmacia Biotech).

**RNA extraction and Real-time PCR**

Total RNA of the cells were extracted using RNeasy Mini Kits (Qiagen, Helden, Germany) according to the manufacturer’s instructions. Three µg RNA of each sample was thereafter reverse-transcribed to first strand cDNA in 20 µl of reaction mixture using a SuperScript™ First-Strand Synthesis System with a RT-PCR kit (Invitrogen, USA). Measurement was performed by a ABI PRISM 9700 HT sequence detection system (Applied Biosystems, Foster City, CA) using a predeveloped Taqman probe/primer combination for TARC. Taqman PCR was performed in 10 ul volume using AmpliTaq Gold polymerase and universal master mix (Applied Biosystems). Threshold cycle numbers
were transformed using the \( \Delta \Delta \text{Ct} \) (threshold cycle) and relative value method, as described by the manufacturer, and were expressed relative to \( \beta \)-actin, which was used as a housekeeping gene by multiplexing single reactions.

**ELISA assay**

The MDC, TARC and IP-10 concentrations of the cell supernatants were determined using commercially available ELISA based assay systems (R & D system, Minneapolis, MN). Assays were performed using the protocols recommended by the manufacturer.

**Statistical analysis**

All data are presented as mean ± SD. One-way analysis of variance was used for all statistical comparisons, and the Student-Newman-Keuls test was conducted for multiple comparisons. A \( P \) value <0.05 was considered significant. SigmaStat software (Jandel Scientific, Erkrath, Germany) was used for all statistical analysis.
Results:

Formoterol and higher doses of salmeterol enhanced MDC expression in THP-1 cells and human primary monocytes

LPS-induced MDC production in THP-1 cells was significantly enhanced in the presence of formoterol (10⁻⁸ M-10⁻⁶ M) after 12 and 24 hours of stimulation (Fig. 1A and 1B). However, formoterol (10⁻⁸ M-10⁻⁶ M) alone could not induce MDC expression in THP-1 cells. Salmeterol could also enhance MDC production in THP-1 cells induced after 12 hours of LPS stimulation but only at the highest concentration (10⁻⁶ M) (Fig. 1A). Similarly, formoterol and only a higher dose of salmeterol (10⁻⁶ M) were found to enhance TNFα-induced MDC expression which was inhibited by budesonide (Fig. 1C). Moreover, the effect of formoterol was seen at the level of gene transcription, since formoterol and salmeterol (at higher dose, 10⁻⁷ M) were able to enhance LPS-induced MDC mRNA expression in THP-1 cells (Fig. 1D). Interestingly, formoterol (10⁻⁹ M-10⁻⁷ M), but not salmeterol (10⁻⁹ M-10⁻⁷ M), could enhance the LPS-induced MDC production in human primary monocytes after 24 hours of LPS stimulation (Fig. 1E).

ICI 118551 reversed, but budesonide suppressed, formoterol- and LPS-induced MDC expression in THP-1 cells

Pretreatment of the cells with ICI 118551 (a selective β2 antagonist) for half an hour could reverse formoterol and LPS-induced MDC expression in THP-1 monocytic cells (Fig.
Formoterol could enhance LPS-induced MDC production in THP-1 cells, therefore we next examined whether budesonide had any effect on the MDC expression. Budesonide could suppress LPS or LPS plus formoterol-induced MDC expression in THP-1 cells (Fig. 2B), suggesting that the inhibitory activity of budesonide on LPS plus formoterol-induced MDC expression of THP-1 cells may be directed against the signaling pathway mobilized by LPS.

Formoterol, salmeterol, fluticasone and budesonide could suppress LPS-induced IP-10 expression in THP-1 cells

Furthermore, formoterol and salmeterol could suppress LPS-induced IP-10 production in THP-1 cells after 24 hours of stimulation (Fig. 3A). Formoterol could also reduce TNFα-induced IP-10 production in THP-1 cells after 24 hours of LPS stimulation (Fig. 3B). Fluticasone and budesonide (Fig. 3C) could suppress the LPS-induced IP-10 production in THP-1 cells. Figure 3D and 3E showed that ICI 118551 (a selective β2 antagonist) could reverse the suppressive effect of formoterol and salmeterol on LPS-induced IP-10 expression in THP-1 monocytic cells.

Formoterol and salmeterol had no effect on LPS-induced MDC and IP-10 expression in THP-1 cell-derived macrophages
Both LABA and corticosteroids are usually administered locally in asthmatic patients, and macrophages are likely to be the targets for modulation. Using THP-1-derived macrophages following in vitro culture of the cells with PMA as a model, the results showed that both formoterol and salmeterol reveal no significant effect on LPS-induced MDC, IP-10 and TNFα expression in THP-1-derived macrophages (Figs. 4A, 4B, 4C).

**Formoterol and salmeterol could suppress the TARC expression in BEAS-2B cells**

When BEAS-2B cells and TARC expression were analyzed, formoterol and salmeterol were shown to be able to suppress the TARC mRNA (Fig 5A) and protein (Fig. 5B) expression in BEAS-2B cells stimulated with a combination of TNFα (50 ng/ml), IL-4 (50 ng/ml) and IFNγ (10 ng/ml). This suppressive effect could be reversed by the addition of ICI118551 (Fig. 5C), and the combination of budesonide and LABA or budesonide only could significantly suppress TARC expression in BEAS-2B cells (Fig. 5D).

**The effects of LABA on Th2-related chemokines may be via NFkB or cAMP pathway**

Further, salmeterol significantly suppressed IκBα expression in THP-1 cells (Fig. 6A), and the addition of an IκBα inhibitor, BAY 11-7085 (5-10 µM), significantly down-regulated LPS-induced MDC production in THP-1 cells (Fig. 6B). These data suggested that salmeterol may enhance LPS-induced MDC expression via its ability to
suppress IkBα expression of THP-1 cells. To examine whether increased cAMP levels could
regulate MDC expression in monocytes and TARC expression in bronchial epithelial cells,
THP-1 cells and BEAS-2B cells, respectively, were pretreated with etazolate (a
phosphodiesterase inhibitor, Calbiochem, Cambridge, MA) one hour before stimulation. As
shown in Fig. 6C, etazolate could enhance LPS-induced MDC expression in THP-1 cells but
suppress TARC expression in BEAS-2B cells stimulated with a combination of TNFα, IL-4
and IFNγ (Fig. 6D). These findings suggest that the chemokine expression in monocytes and
bronchial epithelial cells may be modulated, at least partially, via the NFκB or β2
receptor-cAMP pathway.

**Formoterol-treated monocytes and BEAS-2B cells enhanced chemotaxis Th2 cells**

Since LABAs have an opposite effect on the production of MDC by monocytes or of TARC
by epithelial cells, analysis of the chemotactic activity of LABA-treated cells on Th2 and
Th1 lymphocytes was performed. The results revealed that formoterol-treated THP-1 cells
and BEAS-2B cells could increase the chemotaxis of the Th2 cells, but suppress that of the
Th1 lymphocytes (Fig. 7).
**Discussion:**

Chemokines are known to be important in the pathophysiology of asthma and allergies; in particular, increased expression of TARC and MDC has been found in the bronchial epithelium of asthmatic patients, and is associated with the recruitment of Th2 cells in the inflammatory airways (17,19). Further, LPS is known to be ubiquitously present in the environment and induces Th1- and Th2-related chemokine expression. Exposure to airborne endotoxin in infancy may protect against asthma by promoting the Th1 response and tolerance to allergens. However, later in life, it adversely affects patients with asthma (20). Salmeterol and formoterol have both been introduced as long-acting bronchodilators for the therapeutic management of asthma. In this study, we showed that formoterol enhances the expression of a Th2-related chemokine, MDC, in LPS-treated monocytes, implying a possible occurrence of Th2-associated inflammation following the formoterol monotherapy via systemic administration. Moreover, both long-acting β2-adrenoceptor agonists were shown to be able to suppress the expression of TARC in bronchial epithelial cells at physiological concentrations. This may be the reason why formoterol and salmeterol should be administered in an inhalation form. Of interest to note, LABAs showed an opposite effect on the production of MDC in monocytes or of TARC in epithelial cells. Also, increased chemotactic activity of Th2 cells was found following the treatment of the cells with LABA.
It is possible, therefore, that the combined use of inhaled LABA and corticosteroid may provide an optimal control of asthma in most patients.

Also, chronic use of β2-agonists might increase the risk of bronchial hyperresponsiveness (BHR) and cause greater airway inflammation resulting in increased BHR and a greater decline in lung function (21). It has been suggested that β2-agonists may also mask the effects of increased airway inflammation (22). β2-adrenergic receptors are present not only on smooth muscle cells but also on neutrophils, lymphocytes, monocytes and macrophages (23). In the present study, relevant drug concentrations mimic the in vivo situation at the airway level (10^{-10} M – 10^{-6} M formoterol and 10^{-8} M budesonide) (24). Formoterol at physiologic concentrations could enhance LPS-induced MDC expression in human monocytes which plays an important role in the pathogenesis of airway inflammation in asthma. Budesonide can block formoterol and LPS-induced MDC expression. Therefore, the relevance of LABA monotherapy in asthma may be questioned, as it is advised that LABA be used in combination with inhaled steroids, while short-acting β2-agonists should only be used without inhaled steroids in very mild intermittent asthma.

Formoterol is more potent and a full agonist relative to salmeterol, a partial agonist (25). By contrast, salmeterol is about two-thirds less efficacious than either formoterol or isoprenaline as an inhibitor of histamine release (25). In the present study, MDC production in monocytes induced by LPS was enhanced significantly by formoterol (10^{-9} M-10^{-7} M).
Only higher doses of salmeterol (10^{-6} M), but not lower doses of salmeterol (10^{-9} M-10^{-7} M), could significantly enhance LPS-induced MDC expression in human monocytes. These results suggest, therefore, that the long-term use of a full LABA may have a risk in developing an allergic inflammation in comparison to the use of a partial LABA. Formoterol is more potent in inducing Th2-related chemokine MDC and suppressing Th1-related chemokine IP-10, suggesting that the affinity in binding to the \beta_2 receptor may be the key point in inducing Th2-, but suppressing Th1-related, chemokines. \beta_2-agonists and budesonide could also suppress IL-1 \beta release in blood monocytes, however, macrophages may exhibit more resistance to the treatment of \beta_2-agonists and budesonide (26). This may explain why monocyte-derived macrophages were not sensitive to the effect \beta_2-agonists on chemokine expression in the present study.

It is known that LABAs act primarily to relax airway smooth muscle via the activation of cAMP, and that additional LABAs’ effects may include the stabilisation of inflammatory cell activity and inhibitory effects on the pulmonary mast cells, correlating with the increase in cAMP levels induced by these agonists (25,27). To examine whether, indeed, increasing cAMP levels could enhance MDC expression in monocytes and suppress TARC expression in bronchial epithelial cells, THP-1 cells and BEAS-2B cells were pretreated with etazolate (a phosphodiesterase inhibitor) two hours before stimulation. Etazolate could enhance LPS-induced MDC expression in THP-1 cells (Fig. 6C) but suppress TARC expression in
BEAS-2B cells (Fig. 6D). These results suggested that the expression of chemokines in monocytes and bronchial epithelial cells may be modulated, at least partially, via the β2 receptor-cAMP pathway. Thus, in addition to their effects on relaxing airway smooth muscle and pulmonary mast cells, LABAs could also modulate Th1- and Th2-related chemokine production via the activation of cAMP.

NFκB activation may be responsible, in part, for increased expression of many inflammatory genes in asthma (28). There have been conflicting evidences regarding the action of cAMP/cAMP-dependent protein kinase (PKA) signaling pathway on NFκB. It is known that PKA activating agents inhibit the NFκB-dependent reporter gene expression induced by the activation of TNFα (29). In macrophage, LPS-stimulated NFκB has been shown to be cooperatively activated by cAMP-dependent and -independent PKA activation (30). In the present study, LABAs could enhance LPS-induced MDC expression in monocytes. ICI 118551, a specific β2 receptor antagonist could reverse this effect. The finding that IkBα expression was suppressed by salmeterol in our study suggests that β2 adrenergic receptor/cAMP pathway activation may enhance LPS-induced NFκB expression via suppressing IkBα expression in monocytes. Within the proximal promoter region, MDC has been identified to contain potential binding sites for NFκB, subunits p50 and p65 (31). Thus, LABAs may suppress IkBα expression and cause translocation of NFκB into the nucleus via increasing cAMP, and subsequently transactivating the promoter of the MDC
gene. In conclusion, through NFκB or β2 adrenergic receptor/cAMP pathway, LABAs may increase Th2-related chemokine expression of monocytes and Th2 cell chemotaxis, but suppress Th2-related chemokine in bronchial epithelial cells. Therefore, LABA may be better administered in an inhalation form and LABA monotherapy may not be a good option for asthma.
References:


Legends:

Figure 1: Pretreatment with formoterol ($10^{-8}$ M-$10^{-6}$ M) and only higher doses of salmeterol ($10^{-6}$ M) could enhance the LPS-induced MDC production in THP-1 cells after 12 hours (A) of LPS stimulation. Formoterol ($10^{-8}$ M-$10^{-6}$ M) could also enhance LPS-induced MDC expression significantly in THP-1 cells after 24 hours of LPS stimulation (B). Formoterol ($10^{-8}$-$10^{-6}$ M) and higher dose of salmeterol ($10^{-6}$ M) enhanced TNF-$\alpha$-induced MDC expression which could be suppressed by budesonide (C). Formoterol more than salmeterol could enhance LPS-induced MDC mRNA expressions in THP-1 cells in a dose-dependent manner (D). Pretreated formoterol, but not salmeterol could enhance the LPS-induced MDC production in human primary monocytes after 24 hours of LPS stimulation (E) (*: $P < 0.05$).

Figure 2: ICI 118551 (selective $\beta_2$ agonist) could reverse formoterol and LPS-induced MDC expression in THP-1 monocytic cells (A). Budesonide could significantly suppress LPS only and LPS plus formoterol-induced MDC expression in THP-1 monocytic cells (B).

Figure 3: Pretreatment with formoterol and salmeterol could suppress LPS-induced IP-10 production in THP-1 cells after 24 hours of LPS stimulation (A). Pretreatment with formeterol could suppress TNF-$\alpha$-induced IP-10 production in THP-1 cells after 24 hours of LPS stimulation (B). Fluticasone and budesonide (C) could suppress the LPS-induced IP-10 production in THP-1 cells after 24 hours of LPS stimulation. ICI 118551 (a selective $\beta_2$
agonist) could reverse the suppressive effect of (D) formoterol and (E) salmeterol on LPS-induced IP-10 expression in THP-1 monocytic cells (*: $P < 0.05$).

**Figure 4:** Effects of formoterol and salmeterol had no effect on LPS-induced MDC (A), IP-10 (B) or TNFα (C) expressions in macrophages.

**Figure 5:** Stimulation with TNFα (50 ng/ml), IL-4 (50 ng/ml) and IFNγ (10 ng/ml) could increase TARC mRNA and protein expression in BEAS-2B cells. Formoterol and salmeterol could suppress the TARC mRNA (A) and protein (B) expressions in BEAS-2B cells. This suppressive effect could be reversed by ICI118551 (C). Combination of budesonide and LABA or budesonide only could significantly suppress TARC expression in BEAS-2B cells (D).

**Figure 6:** Salmeterol ($10^{-6}$M) could significantly suppress IkBα expression in THP-1 cells (A). BAY 11-7085 could significantly suppress LPS-induced MDC production in THP-1 cells (B). Etazolate also could significantly enhance LPS-induced MDC production in THP-1 cells and down-regulate TARC expression in BEAS-2B cells (C) and (D) (*: $P < 0.05$).

**Figure 7:** Formoterol-treated THP-1 cells increased Th2 and inhibited Th1 lymphocyte chemotaxis significantly (*: $P < 0.05$). Formoterol-treated BEAS-2B cells only slightly
decreased Th2 lymphocyte chemotactic activity ($P > 0.05$) and had no effect on Th1 cell chemotaxis. Formoterol-treated THP-1 cells and BEAS-2B cells could increase Th2 and suppress Th1 lymphocyte chemotactic activity (*: $P < 0.05$).
Figure

Figure 1:

(A)

LPS 0.2 mcg/ml

Formoterol
Salmeterol

(B)

LPS 0.2 mcg/ml

Formoterol
(C)

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MDC (pg/mL)

- Formoterol
- Salmeterol

* P < 0.05
** P < 0.01
(D) LPS 0.2 mcg/ml

![Bar chart showing MDC mRNA/β-actin levels with asterisks indicating statistical significance for Formoterol and Salmeterol at different concentrations.](chart1)

(E) LPS 0.2 mcg/ml

![Bar chart showing MDC levels with asterisks indicating statistical significance for Formoterol and Salmeterol at different concentrations.](chart2)
Figure 2:

(A) MDC (pg/ml)

LPS 0.2 mcg/ml

Formoterol $10^{-8}$ M

ICI 118551

(B) MDC (pg/ml)

LPS 0.2 mcg/ml

Formoterol $10^{-8}$ M

Budesonide

*(significant differences)*
Figure 3:

(A)

![Graph showing the effect of LPS 0.2 mcg/ml on IP-10 levels with Formoterol and Salmeterol.]

(B)

![Graph showing the effect of TNFα 20 ng/ml on IP-10 levels with Formoterol.]

* indicates significance.
(C) LPS 0.2 mcg/ml

![Graph C](image)

(D) LPS 0.2 mcg/ml

![Graph D](image)
Control

ICI 118551

Salmeterol 10^{-8} M

LPS 0.2 mcg/ml

IP-10 (pg/ml)

0 200 400 600 800 1000 1200 1400

Control 10^{-6} ICI 118551 10^{-5} M

*
Figure 4:

(A)  LPS 0.2 mcg/ml

(B)  LPS 0.2 mcg/ml

(C)  LPS 0.2 mcg/ml
Figure 5:

(A) TARC mRNA/\beta\text{-actin}

(B) TARC (pg/ml)

\begin{align*}
\text{TNF}_\alpha \ + \ IL-4 \ + \ IFN_\gamma \\
\text{(-)} \quad \text{(-)} \quad 10^{-9} \quad 10^{-8}
\end{align*}
Figure 6:

(A)

IkBα/β-actin ratio

![IkBα and β-actin ratio graph]

LPS 0.2 mcg/ml
- + + + + +
Salmeterol
- - 10^-6 10^-7 10^-6 uM

(B)

LPS 0.2 mcg/ml

![MDC (pg/ml) graph]

MDC (pg/ml)

Control 5 10 μM

BAY 11-7085
(C) LPS 0.2 mcg/ml

Etazolate

MDC (pg/ml)

0

2000

4000

6000

8000

10000

12000

10^-6 10^-5 M

(D) TNFα +IL-4+IFNγ

Etazolate

TARC (pg/ml)

0

20

40

60

80

100

120

140

160

10^-6 10^-5 M
Figure 7:

![Graph showing cell numbers of chemotaxis for different cell types and treatments.](image-url)

- **Control THP-1**: 
- **BEAS-2B**: 
- **THP-1+ BEAS-2B**: 
- **THP-1+ Formoterol**: 
- **BEAS-2B+ Formoterol**: 

- **Th1** and **Th2** cell types are indicated.

Statistical significance is marked with asterisks (*).