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

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ABSTRACT: It is unknown whether formoterol and salmeterol, two long-acting β_2-adrenoreceptor agonists, have regulatory functions in the production of T-helper cell (Th) type 2- and Th1-related chemokines by monocytes and bronchial epithelial cells.

In the present study, the effects of formoterol and salmeterol on lipopolysaccharide (LPS)-induced expression of the Th2-related chemokine macrophage-derived chemokine (MDC; CCL22) and the Th1-related chemokine interferon-γ-inducible protein (IP)-10 (CXCL10) were investigated in a monocytic cell line, THP-1, and in human primary monocytes. In addition, their effects on the expression of the Th2-related chemokine thymus- and activation-regulated chemokine (TARC; CCL17) were evaluated in an epithelial cell line, BEAS-2B.

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 selective β_2-adrenoreceptor antagonist, ICI-118551. Formoterol- and LPS-induced MDC expression was inhibited by budesonide.

Both long-acting β_2-adrenoreceptor agonists suppressed thymus- and activation-regulated chemokine expression in bronchial epithelial cells mediated *via* β_2-adrenoreceptors. Formoterol at physiological concentrations could suppress lipopolysaccharide-induced T-helper cell type 1-related chemokine (interferon-γ-inducible protein-10) but enhance T-helper cell type 2-related chemokine (macrophage-derived chemokine) expression in human monocytes. Long-acting β_2-adrenoreceptor agonists may increase T-helper cell type 2-related chemokine expression in monocytes and T-helper cell type 2 recruitment and, therefore, long-acting β_2-adrenoreceptor agonist monotherapy may not be an appropriate therapeutic option for asthma.

KEYWORDS: β_2-Adrenoreceptor agonist, bronchial epithelial cells, bronchodilators, CC chemokines, macrophage, monocyte

Asthma is a chronic inflammatory disorder of the airway [1]. Infiltration of the airways by T-helper cell (Th) type 2 lymphocytes, eosinophils and other inflammatory cells is a well-recognised 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 and 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. Interferon (IFN)-γ-inducible protein (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 IFN-γ [4] and is also upregulated in allergic pulmonary inflammation [5]. IP-10 is more highly expressed in airway smooth muscle of subjects with asthma when compared with healthy control subjects, 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

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4-bearing Th2 cells in allergen-induced inflammation [7]. Increased levels of plasma MDC and TARC have been found in children with acute asthma, but their levels decrease after ketotifen treatment [8].

β_2-Adrenoreceptor agonists are firstline drugs for the treatment of acute asthma due to their potent and rapid bronchodilatory effects. Inhaled long-acting β_2-adrenoreceptor agonists (LABAs) are generally combined with corticosteroids to control asthma, and are considered to be smooth muscle relaxants. While their anti-inflammatory properties are still a matter of debate, their anti-inflammatory effects are suggested by inhibitory effects 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 the pro-inflammatory cytokines interleukin (IL)-6, IL-8 and tumour necrosis factor (TNF)-α in a variety of cell types. For example, formoterol has been shown to be able to suppress lipopolysaccharide (LPS)-induced IL-6 expression in a mouse model, but to enhance the expression of IL-8 in bronchial epithelial cells [11, 12]. Also, salmeterol has been shown to inhibit TNF-α secretion in LPS-activated THP-1 cells [13] and to suppress the immunoglobulin E-dependent release of TNF-α from human skin mast cells [14]. WALLIN *et al*. [15] recently reported the inhibition of eosinophil infiltration by formoterol in subjects with asthma. Additionally, inhaled LABAs and corticosteroids give optimal control of asthma in most patients, and two fixed-combination inhalers (salmeterol/fluticasone and formoterol/budesonide) have increasingly been used as convenient controllers in patients with persistent asthma. Thus, there is a strong scientific rationale for the combination of these two drug classes [16].

In the present study, the authors investigated whether formoterol and salmeterol have regulatory effects on the expression of the Th2-related chemokines MDC and TARC and the Th1-related chemokine IP-10 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, USA) was cultured in RPMI 1640 medium (Sigma-Aldrich Co., St Louis, MO, USA) supplemented with 10% foetal bovine serum, 100 U·mL^{-1} penicillin and 100 μg·mL^{-1} streptomycin at 37°C with 5% CO_2 in a humidified incubator. THP-1 cells were incubated at 37°C with 3.2×10^{-7} M phorbol myristate acetate (PMA; Sigma-Aldrich Co.). After incubating with PMA for 24 h, THP-1 cells had differentiated into macrophage-like cells. Cells were centrifuged and resuspended in fresh media in 24-well plates at a concentration of 10^6 cells·mL^{-1} for 24 h before experimental use. The cells were pre-treated with formoterol (10^{-10}–10^{-7} M; AstraZeneca, Alderley Park, UK), salmeterol (10^{-10}–10^{-5} M; GlaxoSmithKline, Brentford, UK) or etazolate (10^{-6}–10^{-5} M; Calbiochem, Cambridge, MA, USA) for 1 h before LPS (0.2 μg·mL^{-1}) or TNF-α (20 ng·mL^{-1}) stimulation. BEAS-2B cells (American Type Culture Collection) were pre-treated with formoterol, salmeterol or etazolate for 1 h before TNF-α (50 ng·mL^{-1}), IL-4 (50 ng·mL^{-1}) and IFN-γ (10 ng·mL^{-1}) stimulation [17]. Cell supernatants were collected after stimulation for 12, 24 and 48 h. In some cases, the cells were pre-treated with the selective β_2-adrenoreceptor antagonist ICI-118551 (Calbiochem), the nuclear factor (NF)-κB pathway inhibitor BAY 11-7085 (Calbiochem), or the corticosteroids fluticasone (GlaxoSmithKline) or budesonide (Sigma-Aldrich Co.), for 30 min before treatment of the cells with formoterol or salmeterol.

Peripheral blood samples were obtained from three healthy individuals who had no personal or family history of allergic diseases. Cord blood was harvested from delivered placentas into a standard blood collection bag with citrate phosphate dextrose anticoagulant. The cord blood was collected from the delivered placenta to avoid any risk to the mother or infant from the collection process. The study protocol was approved by the Institutional Review Board of Kaohsiung Medical University Hospital (Kaohsiung, Taiwan, Republic of China). Informed consent was obtained from three healthy adult volunteers and each parent of the cord blood donors. Blood samples were diluted with an equal volume of PBS. Peripheral blood mononuclear cells were isolated by density gradient centrifugation (Lymphoprep™; Axis-Shield PoC, Oslo, Norway), and monocytes were isolated by magnetic bead sorting with the anti-CD14 monoclonal antibody (mAb) MACS® (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany).

In vitro *polarisation 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-Paque™ (GE Healthcare, Uppsala, Sweden). The CD4+ lymphocytes were further purified by magnetic bead sorting with an anti-CD4 mAb (MACS®; Miltenyi Biotec GmbH). The cells were polarised as previously described [18]. Th2 cultures were supplemented with 10 μg·mL^{-1} anti-IL-12 (R&D Systems, Minneapolis, MN, USA) and 10 ng·mL^{-1} IL-4 (R&D Systems). T-cells were cultured with 5 ng·mL^{-1} IL-12 and 10 μg·mL^{-1} anti-IL-4 (R&D Systems) for Th1 polarisation. After 48 h of priming, 5 ng·mL^{-1} IL-2 (R&D Systems) was added to the cultures. The cells were cultured in the presence of IL-2 alone without the addition of any polarising cytokines. After 7 days of polarisation, IFN-γ and IL-4 cytokine production was determined by ELISA (R&D Systems; data not shown).

Chemotactic assay

Chemotaxis of Th1 and Th2 cells was measured using a 24-well Micro Chemotaxis Transwell (Corning Costar, Cambridge, MA, USA). Th1 and Th2 cells were resuspended at 3×10^5 cells·mL^{-1} and loaded onto the upper chamber of the Micro Chemotaxis chamber. The supernatants of LPS-treated THP-1 cells or IL-4-, TNF-α- and IFN-γ-treated BEAS-2B cells were added to the lower chamber. In some cases, both kinds of supernatant were mixed with a 1:1 ratio. The lower and upper chambers were separated by a polycarbonate membrane (5-μm pore size). Th1 and Th2 cells were left to transmigrate for 3 h at 37°C in a humidified atmosphere with 5% CO_2. After incubation for 3 h, the number of migrated Th2 cells in the lower compartment was determined by counting the cells under a light microscope. The percentage of inhibition was 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^{-1}) and lysed 1 h later with an equal volume (150 μL) of ice-cold lysis buffer. After centrifugation at 13,000 × *g* for 15 min, equal amounts of cell lysates from each experimental condition were analysed by Western blot with anti-IκB-α and anti-β-actin antibodies (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). Immunoreactive bands were visualised using horseradish peroxidase-conjugated secondary antibodies and the enhanced chemiluminescence system (Amersham ECL™; GE Healthcare).

RNA extraction and real-time PCR

Total RNA was extracted using RNeasy Mini Kits (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. From each sample, 3 μg RNA was reverse-transcribed to produce first-strand cDNA in a 20 μL reaction mixture using a SuperScript™ First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). Measurement was performed by an ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using a pre-designed TaqMan® probe/primer combination for TARC (Protech Technology, Taipei, Taiwan). TaqMan® PCR was performed in a 10 μL volume using AmpliTaq Gold® polymerase and universal master mix (Applied Biosystems). Threshold cycle numbers were transformed using the comparative threshold cycle and relative value method, as described by the manufacturer (Applied Biosystems), and were expressed relative to β-actin, which was used as a housekeeping gene by multiplexing single reactions.

ELISA assay

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

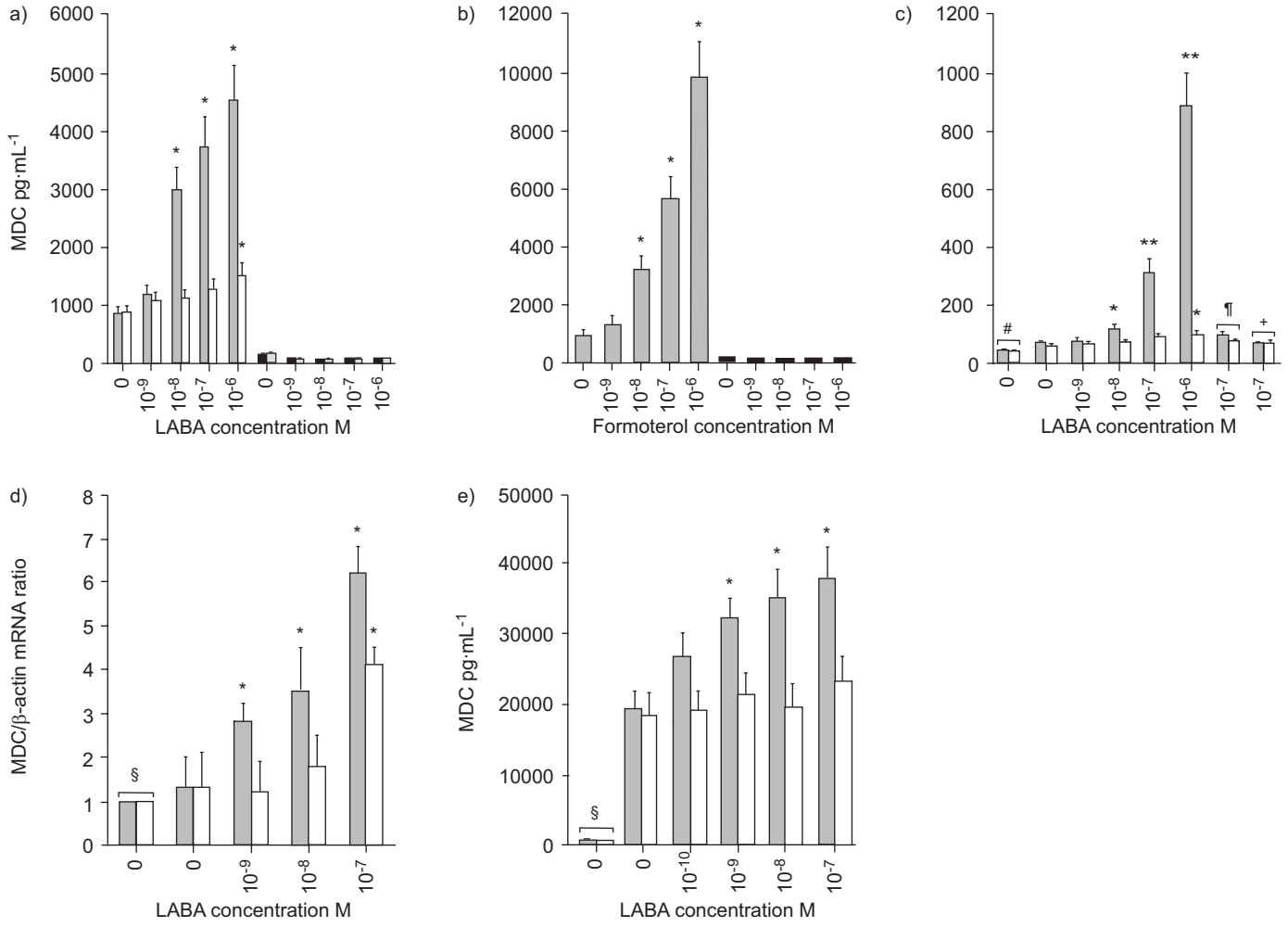

FIGURE 1. a) Pre-treatment with the long-acting β_2-adrenoreceptor agonists (LABAs) formoterol (■ and ■) at 10^{-8}–10^{-6} M and salmeterol (□ and ■) at the high dose of 10^{-6} M enhanced the lipopolysaccharide (LPS)-induced macrophage-derived chemokine (MDC) production in the human monocytic cell line THP-1 after 12 h of 0.2 μg·mL^{-1} LPS stimulation (■ and □). b) Formoterol (10^{-8}–10^{-6} M) also enhanced LPS-induced MDC expression significantly in THP-1 cells after 24 h of LPS stimulation (■). c) Formoterol (10^{-8}–10^{-6} M) and the high dose of salmeterol (10^{-6} M) enhanced tumour necrosis factor (TNF)-α-induced MDC expression in THP-1 cells, and this enhanced expression was suppressed by budesonide. d) Formoterol, more than salmeterol, enhanced LPS-induced MDC mRNA expression (relative to β-actin expression) in THP-1 cells in a dose-dependent manner. e) Pre-treatment with formoterol, but not salmeterol, enhanced the LPS-induced MDC production in human primary monocytes after 24 h of LPS stimulation. ■: no LPS; ■: no LPS. #: no TNF-α; ¶: budesonide at 10^{-6} M; +: budesonide at 10^{-5} M; §: no LPS. *: $p<0.05$; **: $p<0.01$.

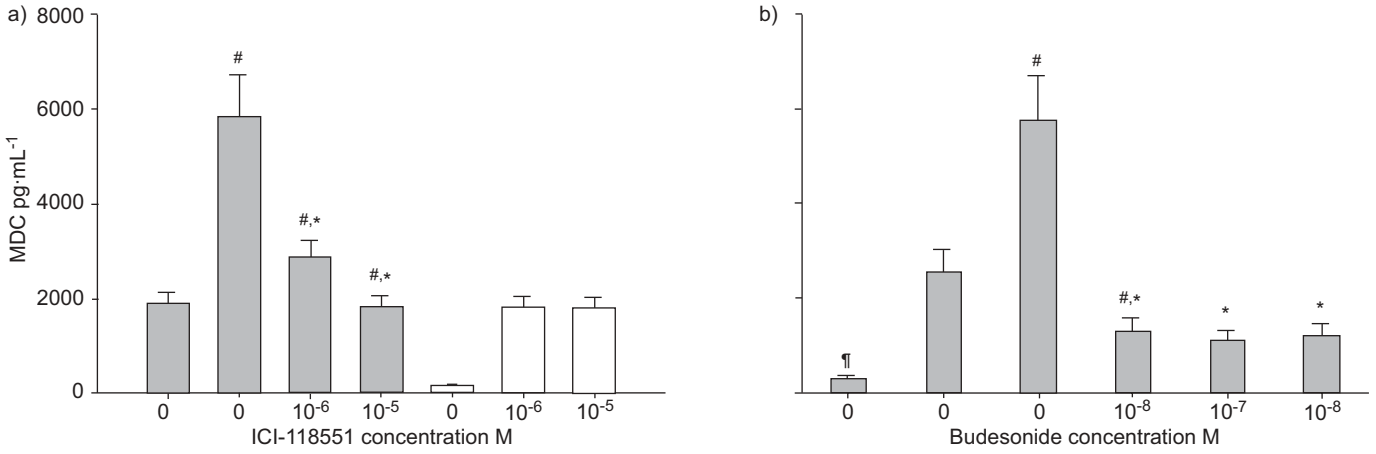

FIGURE 2. a) ICI-118551 (a selective β_2-adrenoreceptor antagonist) reversed formoterol+lipopolysaccharide (LPS)-induced macrophage-derived chemokine (MDC) expression in the human monocytic cell line THP-1. b) Budesonide significantly suppressed LPS- and LPS+formoterol-induced MDC expression in THP-1 cells. ■: LPS stimulated; □: no LPS. #: formoterol at 10^{-8} M; ¶: no LPS. *: $p<0.05$.

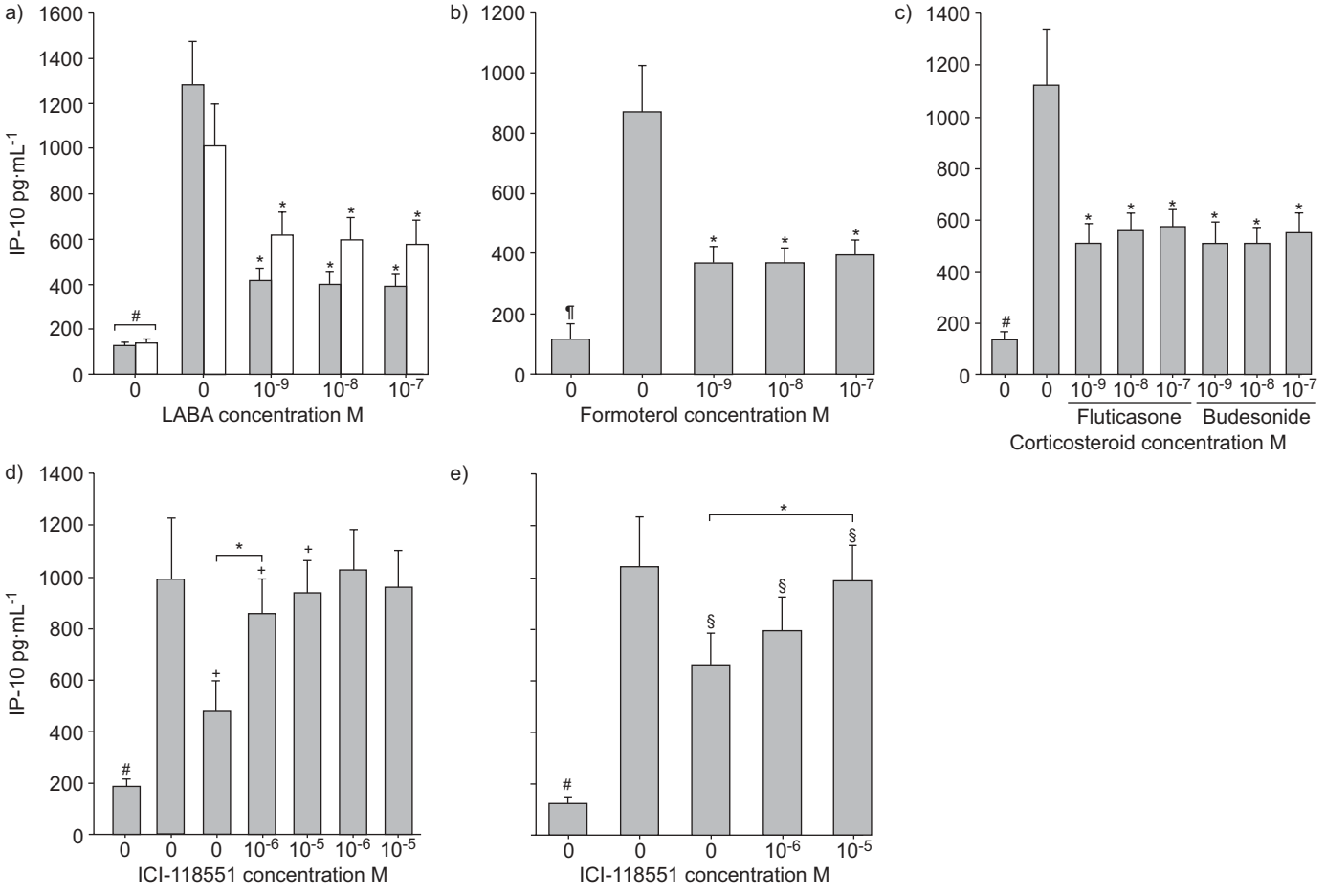

FIGURE 3. a) Pre-treatment with the long-acting β_2-adrenoreceptor agonists (LABAs) formoterol (■) and salmeterol (□) suppressed lipopolysaccharide (LPS)-induced interferon-γ-inducible protein (IP)-10 production in the human monocytic cell line THP-1 after 24 h of 0.2 μg·mL^{-1} LPS stimulation. b) Pre-treatment with formoterol suppressed tumour necrosis factor (TNF)-α-induced IP-10 production in THP-1 cells after 24 h of 20 ng·mL^{-1} TNF-α stimulation. c) The corticosteroids fluticasone and budesonide suppressed the LPS-induced IP-10 production in THP-1 cells after 24 h of LPS stimulation. ICI-118551 (a selective β_2-adrenoreceptor antagonist) reversed the suppressive effect of d) formoterol and e) salmeterol on LPS-induced IP-10 expression in THP-1 cells. #: no LPS; ¶: no TNF-α; +: formoterol at 10^{-8} M; §: salmeterol at 10^{-8} M. *: $p<0.05$.

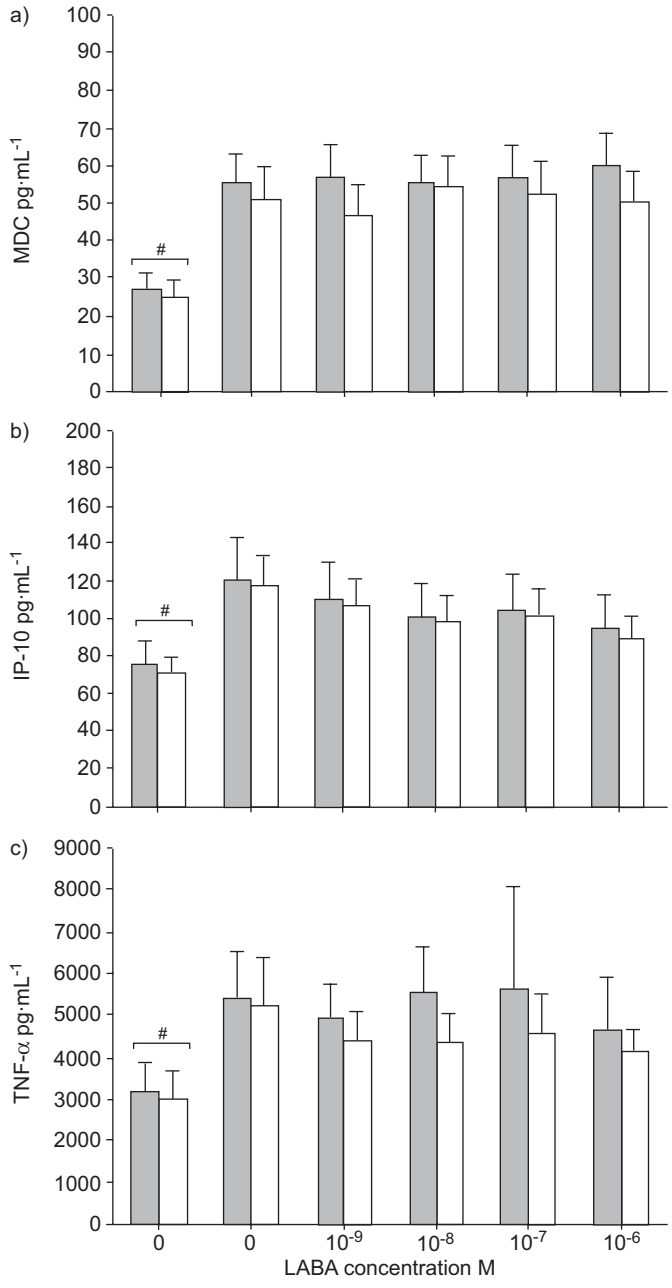

FIGURE 4. The long-acting β_2-adrenoreceptor agonists (LABAs) formoterol (■) and salmeterol (□) had no effect on lipopolysaccharide (LPS)-induced expression in macrophages of a) macrophage-derived chemokine (MDC), b) interferon-γ-inducible protein (IP)-10 or c) tumour necrosis factor (TNF)-α. #: no LPS.

Statistical analysis

All data are presented as mean±SD. One-way ANOVA was used for all statistical comparisons, and the Student–Newman–Keuls test was conducted for multiple comparisons. A p-value <0.05 was considered statistically significant.

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}–10^{-6} M) after 12 and 24 h of stimulation (fig. 1a and b). However, formoterol (10^{-8}–10^{-6} M) alone could not induce MDC expression in THP-1 cells. Salmeterol also enhanced MDC production in THP-1 cells induced after 12 h 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 TNF-α-induced MDC expression, and this enhanced expression was inhibited by budesonide (fig. 1c). Moreover, the effect of formoterol was seen at the level of gene transcription, since formoterol and salmeterol (at 10^{-7} M) enhanced LPS-induced MDC mRNA expression in THP-1 cells (fig. 1d). Interestingly, formoterol (10^{-9}–10^{-7} M) but not salmeterol (10^{-9}–10^{-7} M) enhanced the LPS-induced MDC production in human primary monocytes after 24 h of LPS stimulation (fig. 1e).

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

Pre-treatment of the cells with ICI-118551 (a selective β_2-adrenoreceptor antagonist) for 30 min reversed formoterol+LPS-induced MDC expression in THP-1 monocytic cells (fig. 2a). Formoterol enhanced LPS-induced MDC production in THP-1 cells; therefore, the effect of budesonide on MDC expression was examined. Budesonide suppressed LPS- or LPS+formoterol-induced MDC expression in THP-1 cells (fig. 2b), suggesting that the inhibitory activity of budesonide on LPS+formoterol-induced MDC expression of THP-1 cells may be directed against the signalling pathway mobilised by LPS.

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

Formoterol and salmeterol suppressed LPS-induced IP-10 production in THP-1 cells after 24 h of LPS stimulation (fig. 3a). Formoterol also reduced TNF-α-induced IP-10 production in THP-1 cells after 24 h of TNF-α stimulation (fig. 3b). Fluticasone and budesonide (fig. 3c) suppressed LPS-induced IP-10 production in THP-1 cells. ICI-118551 reversed the suppressive effect of formoterol and salmeterol on LPS-induced IP-10 expression in THP-1 monocytic cells (fig. 3d and e).

Formoterol and salmeterol had no effect on LPS-induced MDC and IP-10 expression in THP-1-derived macrophages

Both LABAs and corticosteroids are usually administered locally in asthmatic patients, and macrophages are likely to be the targets for modulation. Using THP-1-derived macrophages, produced by *in vitro* culture of the cells with PMA, neither formoterol nor salmeterol had a significant effect on LPS-induced MDC, IP-10 or TNF-α expression (fig. 4).

Formoterol and salmeterol suppressed TARC expression in BEAS-2B cells

When TARC expression in BEAS-2B cells was analysed, 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 TNF-α (50 ng·mL^{-1}), IL-4 (50 ng·mL^{-1}) and IFN-γ (10 ng·mL^{-1}). This suppressive effect was reversed by the addition of ICI-118551 (fig. 5c). Both budesonide alone and the combination of budesonide and LABA significantly suppressed TARC expression in BEAS-2B cells (fig. 5d).

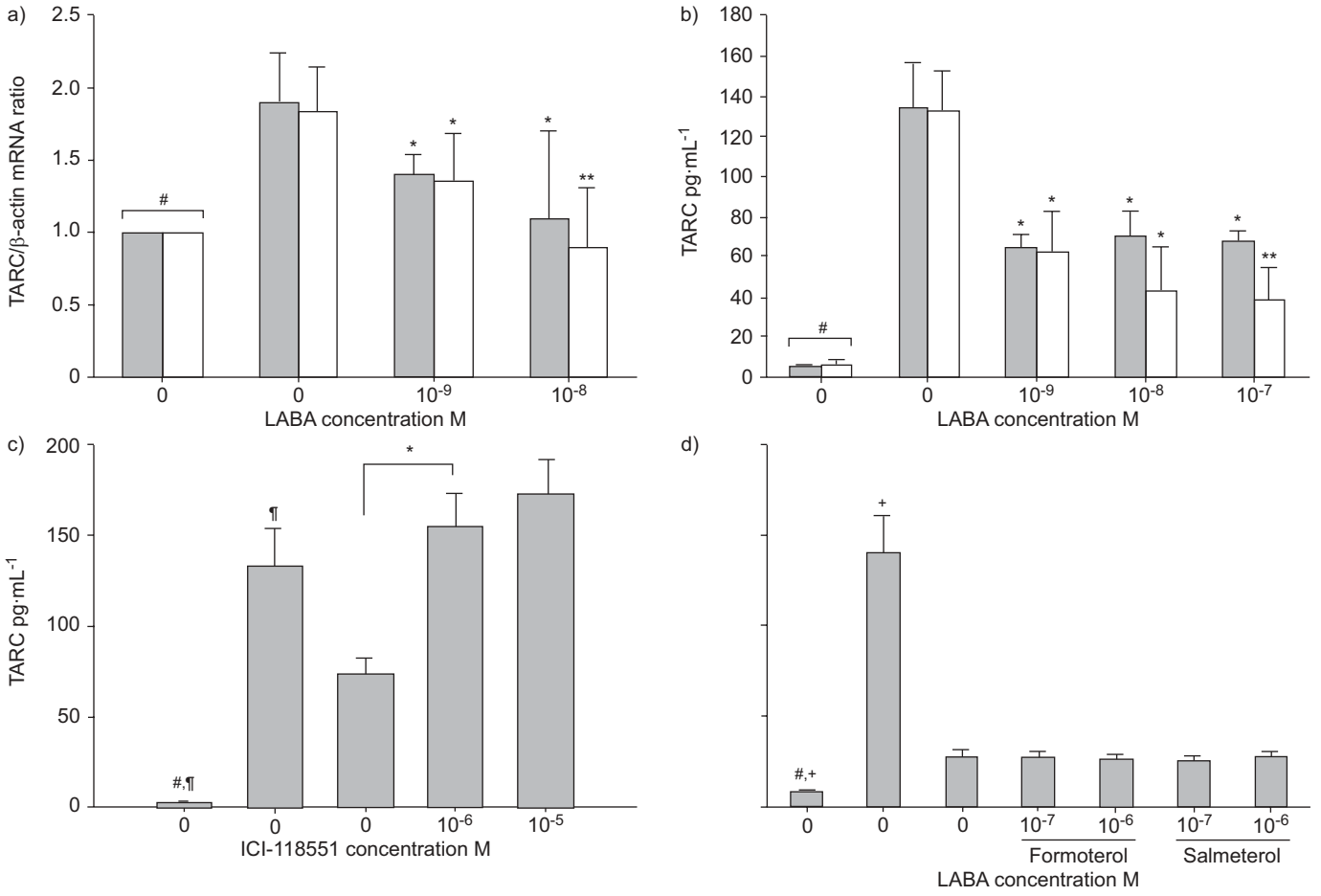

FIGURE 5. Stimulation with tumour necrosis factor (TNF)-α (50 ng·mL^{-1}), interleukin (IL)-4 (50 ng·mL^{-1}) and interferon (IFN)-γ (10 ng·mL^{-1}) increased thymus- and activation-regulated chemokine (TARC) mRNA and protein expression in the bronchial epithelial cell line BEAS-2B. The long-acting β_2-adrenoreceptor agonists (LABAs) formoterol (■) and salmeterol (□) suppressed this induction in BEAS-2B cells of a) TARC mRNA expression (relative to β-actin expression) and b) TARC protein expression. c) The suppressive effect of 10^{-8} M formoterol on TNF-α-, IL-4- and IFN-γ-induced TARC protein expression could be reversed by ICI-118551, a selective β_2-adrenoreceptor antagonist. d) Both 10^{-8} M budesonide alone and the combination of budesonide and LABA significantly suppressed TNF-α-, IL-4- and IFN-γ-induced TARC expression in BEAS-2B cells. $^{\#}$: no TNF-α, IL-4 or IFN-γ; ¶: no formoterol; $^{+}$: no budesonide. *: $p<0.05$; **: $p<0.01$.

The effects of LABAs on Th2-related chemokines may be via the NF-κB or cyclic adenosine monophosphate pathways

Salmeterol significantly suppressed expression of the NF-κB inhibitory subunit, IκB-α, in THP-1 cells (fig. 6a and b), and the addition of an inhibitor of IκB-α degradation, BAY 11-7085 (5–10 μM), significantly downregulated LPS-induced MDC production in THP-1 cells (fig. 6c). These data suggested that salmeterol may enhance LPS-induced MDC expression *via* its ability to suppress IκB-α expression in THP-1 cells. To examine whether increased cyclic adenosine monophosphate (cAMP) levels could regulate MDC expression in monocytes and TARC expression in bronchial epithelial cells, THP-1 cells and BEAS-2B cells, respectively, were pre-treated with etazolate (a phosphodiesterase inhibitor) for 1 h before stimulation. Etazolate enhanced LPS-induced MDC expression in THP-1 cells (fig. 6d) but suppressed TARC expression in BEAS-2B cells stimulated with a combination of TNF-α, IL-4 and IFN-γ (fig. 6e). These findings suggest that the chemokine expression in monocytes and bronchial epithelial cells may be modulated, at least partially, *via* the NF-κB or β_2-adrenoreceptor-cAMP pathways.

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

Since LABAs have an opposite effect on the production of MDC by monocytes and 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 increased the chemotaxis of the Th2 cells but suppressed 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]. Furthermore,

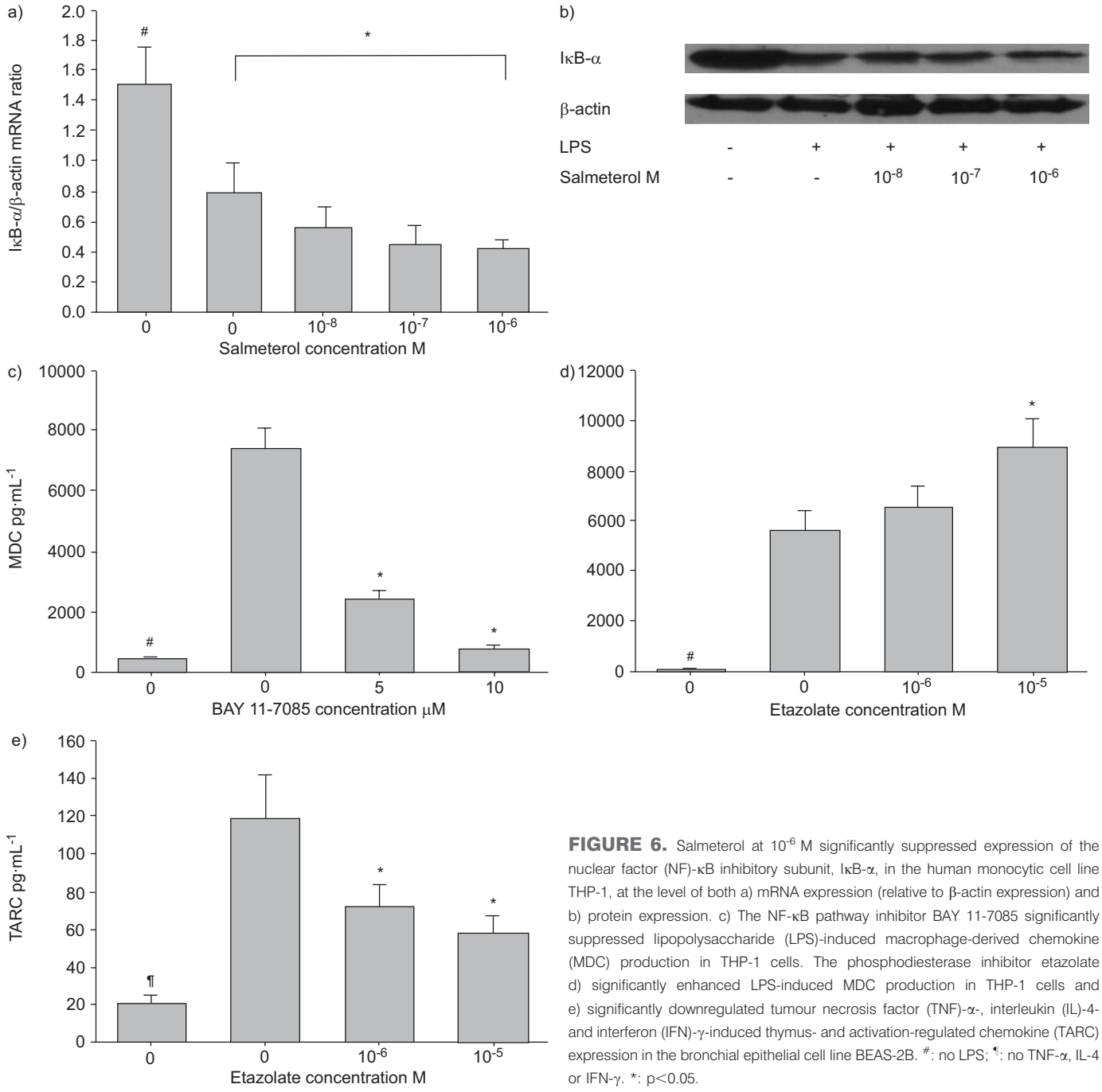

FIGURE 6. Salmeterol at 10^{-6} M significantly suppressed expression of the nuclear factor (NF)-κB inhibitory subunit, IκB-α, in the human monocytic cell line THP-1, at the level of both a) mRNA expression (relative to β-actin expression) and b) protein expression. c) The NF-κB pathway inhibitor BAY 11-7085 significantly suppressed lipopolysaccharide (LPS)-induced macrophage-derived chemokine (MDC) production in THP-1 cells. The phosphodiesterase inhibitor etazolate d) significantly enhanced LPS-induced MDC production in THP-1 cells and e) significantly downregulated tumour necrosis factor (TNF)-α-, interleukin (IL)-4- and interferon (IFN)-γ-induced thymus- and activation-regulated chemokine (TARC) expression in the bronchial epithelial cell line BEAS-2B. #: no LPS; ¶: no TNF-α, IL-4 or IFN-γ. *: $p<0.05$.

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, it adversely affects asthma patients later in life [20]. Salmeterol and formoterol have both been introduced as long-acting bronchodilators for the therapeutic management of asthma. In the present study, formoterol was shown to enhance the expression of a Th2-related chemokine, MDC, in LPS-treated monocytes, implying a possible occurrence of Th2-associated inflammation following formoterol monotherapy *via* systemic administration. Moreover, both LABAs 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. Notably, LABAs showed opposite effects on the production of MDC in monocytes and of TARC in epithelial cells. Also, increased chemotactic activity of Th2 cells was found following 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.

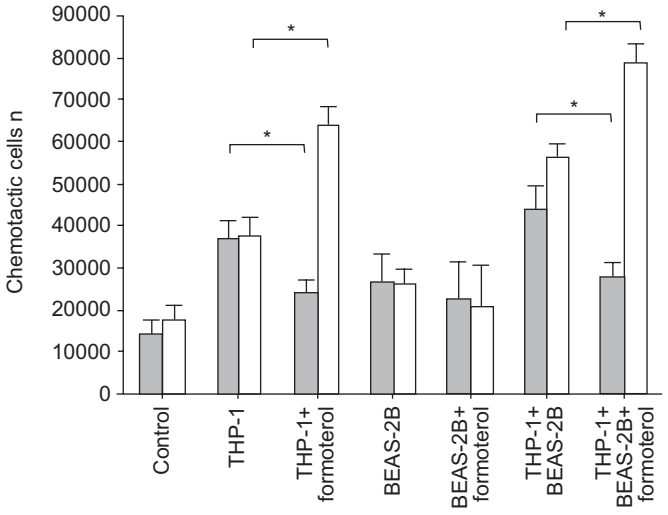

FIGURE 7. Formoterol-treated THP-1 cells significantly increased T-helper cell (Th) type 2 (□) and inhibited Th1 (■) lymphocyte chemotaxis. Formoterol-treated BEAS-2B cells only slightly decreased Th2 lymphocyte chemotactic activity and had no effect on Th1 cell chemotaxis. A combination of formoterol-treated THP-1 cells and BEAS-2B cells increased Th2 and suppressed Th1 lymphocyte chemotactic activity. *: $p<0.05$.

Additionally, chronic use of β_2-adrenoreceptor 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 β_2-adrenoreceptor agonists may also mask the effects of increased airway inflammation [22]. β_2-Adrenoreceptors are present not only on smooth muscle cells but also on neutrophils, lymphocytes, monocytes and macrophages [23]. In the present study, relevant drug concentrations mimicked the *in vivo* situation at the airway level (10^{-10}–10^{-6} M formoterol and 10^{-8} M budesonide) [12]. Formoterol at physiological concentrations enhanced LPS-induced MDC expression in human monocytes, which plays an important role in the pathogenesis of airway inflammation in asthma. Budesonide blocked formoterol and LPS-induced MDC expression. Therefore, the relevance of LABA monotherapy in asthma may be questioned, as it is advised that LABAs be used in combination with inhaled steroids, while short-acting β_2-adrenoreceptor agonists should only be used without inhaled steroids in very mild intermittent asthma.

Formoterol is more potent and a full agonist, compared with salmeterol, which is a partial agonist [24]. In addition, salmeterol is approximately two-thirds less efficacious than either formoterol or isoprenaline as an inhibitor of histamine release [24]. In the present study, MDC production in monocytes induced by LPS was enhanced significantly by formoterol (10^{-9}–10^{-7} M). Only higher doses of salmeterol (10^{-6} M), but not lower doses (10^{-9}–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 higher a risk of causing allergic inflammation in comparison with the use of a partial LABA. Formoterol is more potent in inducing the Th2-related chemokine MDC and suppressing the Th1-related chemokine IP-10, suggesting that the affinity for binding to the β_2-adrenoreceptor may be the key point in inducing Th2- but suppressing Th1-related chemokines. β_2-Adrenoreceptor agonists and budesonide could also suppress IL-1β release in blood monocytes; however, macrophages may exhibit more resistance to treatment with β_2-adrenoreceptor agonists and budesonide [25]. This may explain why monocyte-derived macrophages were not sensitive to the effect of LABAs 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 effects of LABAs 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 [24, 26]. 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 pre-treated with the phosphodiesterase inhibitor etazolate for 1 h before stimulation. Etazolate enhanced LPS-induced MDC expression in THP-1 cells (fig. 6d) but suppressed TARC expression in BEAS-2B cells (fig. 6e). These results suggest that the expression of chemokines in monocytes and bronchial epithelial cells may be modulated, at least partially, *via* the β_2-adrenoreceptor-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.

NF-κB activation may be responsible, in part, for increased expression of many inflammatory genes in asthma [27]. There has been conflicting evidence regarding the action of the cAMP/cAMP-dependent protein kinase (PKA) signalling pathway on NF-κB. It is known that PKA activating agents inhibit the NF-κB-dependent reporter gene expression induced by the activation of TNF-α [28]. In macrophages, LPS-stimulated NF-κB has been shown to be cooperatively activated by cAMP-dependent and -independent PKA activation [29]. In the present study, LABAs enhanced LPS-induced MDC expression in monocytes. ICI-118551, a selective β_2-adrenoreceptor antagonist, reversed this effect. The finding that IκB-α expression was suppressed by salmeterol in the present study suggests that β_2-adrenoreceptor-cAMP pathway activation may enhance LPS-induced NF-κB expression *via* suppression of IκB-α expression in monocytes. Within the proximal promoter region of MDC, potential binding sites for NF-κB subunits p50 and p65 have been identified [30]. Thus, LABAs may suppress IκB-α expression and cause translocation of NF-κB into the nucleus by increasing cAMP and subsequently transactivating the promoter of the MDC gene.

In conclusion, through the nuclear factor-κB or the β_2-adrenoreceptor-cyclic adenosine monophosphate pathways, long-acting β_2-adrenoreceptor agonists may increase T-helper cell type 2-related chemokine expression of monocytes and T-helper cell type 2 chemotaxis, but suppress T-helper cell type 2-related chemokine expression in bronchial epithelial cells. Therefore, long-acting β_2-adrenoreceptor agonists may be better administered in an inhalation form and long-acting β_2-adrenoreceptor agonist monotherapy may not be a good option for asthma.

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