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Human lung and monocyte-derived macrophages differ with regard to the effects of β2-adrenoceptor agonists on cytokine release

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Abstract

Background: β2-adrenoceptor agonists have been shown to reduce the lipopolysaccharide (LPS)-induced cytokine release by human monocyte-derived macrophages (MDMs). We compare the expression of β2-adrenoceptors and the inhibitory effect of formoterol and salmeterol on the LPS-induced release of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and a range of chemokines (CCL2, 3, 4, and IL-8) by human lung macrophages (LMs) and MDMs.

Methods: LMs were isolated from patients undergoing resection and MDMs were obtained from blood monocytes in the presence of GM-CSF. LMs and MDMs were incubated in the absence or presence of formoterol or salmeterol prior to stimulation with LPS. The effects of formoterol were also assessed in the presence of the phosphodiesterase inhibitor roflumilast.

Results: LPS-induced cytokine production was higher in LMs than in MDMs. Salmeterol and formoterol exerted an inhibitory effect on the LPS-induced production of TNF-α, IL-6, CCL2, CCL3, and CCL4 in MDMs. In contrast, the β2-adrenoceptor agonists were devoid of any effect on LMs - even in the presence of roflumilast. The expression of β2-adrenergic receptors was detected on Western blots in MDMs but not in LMs.

Conclusions: Concentrations of β2-adrenoceptor agonists that cause relaxation of the human bronchus can inhibit cytokine production by LPS-stimulated MDMs but not by LMs.

Keywords: β2-adrenoceptor, Cytokines, Lipopolysaccharide, Lung macrophage, Monocyte-derived macrophage

Background

Pollens, house dust mites (HDMs), and cat dander are major triggers in allergic respiratory diseases such as asthma [1–3]. Air pollution is also associated with the acute worsening of pre-existing asthma and chronic obstructive pulmonary disease (COPD) and with progression from asthma to COPD [4, 5].

In addition to its well-characterized involvement in the response to lipopolysaccharide (LPS), toll-like receptor 4 (TLR4) is involved in the airways’ response to various allergens (e.g. ragweed pollen, house dust extract, and cat dander) and many air pollutants including particulate matter and their components other than allergens and LPS, such as viruses and fungal spores [6–9]. Particles that are less than 5 μm in size may gain access to the lower airways and alveoli, where they encounter macrophages (which account for more than 80% of the leukocyte population) [9]. LPS-mediated activation of macrophages causes the release of cytokines (tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and chemokines such as CCL2, CCL3, CCL4, and CXCL8 (IL-8)). This release contributes to airway inflammation by increasing the recruitment of inflammatory cells [10]. Recent research
has highlighted the role of neutrophil recruitment in the response to allergen exposure and the subsequent development of allergen sensitization and inflammation [11].

In murine models of LPS-induced lung inflammation, formoterol and salmeterol reduce the recruitment of neutrophils to the lung and inhibit the release of pro-inflammatory mediators [12, 13]. We recently described the anti-inflammatory effect of the long-acting β₂-adrenoceptor agonist (LABA) olodaterol on (i) murine and guinea pig models of cigarette smoke- and LPS-induced lung inflammation, and (ii) LPS-induced cytokine release from explants of human lung parenchyma [14]. In a clinical setting, salmeterol also reduces neutrophil influx, neutrophil degranulation and TNF-α release after LPS inhalation by healthy individuals [15].

β₂-adrenoceptors are widely expressed throughout the lung [16, 17], and are found on epithelial and bronchial smooth muscle cells, endothelial and vascular smooth muscle cells, and pneumocytes [18, 19]. The β₂-adrenoceptors expressed by airway smooth muscle are involved in the relaxant effect of β₂-adrenoceptor agonists. Moreover, β₂-adrenoceptors are expressed on inflammatory cells, such as neutrophils, monocytes/macrophages and lymphocytes [20–23]. With respect to the monocyte/macrophage lineage, β₂-adrenoceptor activation was shown to variably reduce the LPS-stimulated release of leukotriene B₄ (LTB₄), TNF-α, IL-1β, IL-8 and CCL3 from human peripheral blood mononuclear cells [24–29]. Formoterol and salmeterol suppressed the LPS-induced release of TNF-α by monocyte-derived macrophages (MDMs) [30]. Clobuterol and terbutaline suppressed the LPS-induced TNF-α and IL-6 release by phorbol-myristate-acetate-differentiated U937 human macrophages [31]. Furthermore, salmeterol reduced the cigarette-smoke-extract-induced release of IL-8 by MDMs [32].

Human monocytes, MDMs and U937 macrophages are all surrogate cell models that do not adequately recapitulate the biology of primary tissue macrophages. Previous studies have identified a large number of differentially expressed proteins [33, 34] and genes [35, 36] when comparing unstimulated monocytes, MDMs and human lung macrophages (LMs). It is noteworthy that the scarce data on the anti-inflammatory effects of β₂-adrenoceptor agonists are much less clear for LMs than for MDMs or monocytes. The non-selective β₂-adrenergic agonist isoprenaline did not alter the zymosan- and IgE-triggered release of the eicosanoids LTB₄ and thromboxane B₂ (TXB₂) [37], whereas high concentrations of salmeterol inhibited the release of TXB₂ in LMs [38]. Neither the short-acting β₂-adrenergic agonists salbutamol and terbutaline nor the LABAs salmeterol and formoterol inhibit the LPS-stimulated release of IL-1β [39]. However, treatment with isoprenaline was associated with an increase in levels of cyclic AMP (cAMP) in LMs via the activation of β₂-adrenergic receptors [22, 40, 41]. Furthermore, other cAMP-elevating agents (adenosine receptor agonists, phosphodiesterase 4 (PDE4) inhibitors, PGE₁/2/4 and forskolin) either increased the cAMP content [22, 40] or had inhibitory effects on LPS-induced cytokine release by LMs [41–44]. During the preparation of the present manuscript, Gill et al. reported on the inhibitory effects of high concentrations of β₂-adrenoceptor agonists on the LPS-induced production of TNF-α and IL-6 by LMs [45].

Hence, the present study was designed to assess and compare the effects of the LABAs formoterol and salmeterol on LPS-stimulated cytokine production and the expression of β₂-adrenoceptors by LMs and MDMs. We selected a LABA concentration range (10⁻¹¹ to 10⁻⁷ M) that relaxes isolated human bronchus [46, 47], and we used an LPS preparation that is selective for TLR4. We assessed the production of TNF-α, IL-6 and three CC chemokines (CCL2, CCL3, and CCL4), levels of which are markedly increased by LPS exposure and inhibited by cAMP-elevating agents [30, 41–45]. Furthermore, we assessed the LABAs’ effects on the LPS-induced production of IL-1β and IL-8, which is only weakly or not altered by various cAMP-elevating agents [30, 39, 42–44].

Methods
Reagents
Penicillin-streptomycin, dimethyl sulfoxide (DMSO), fetal calf serum (FCS), LPS from Escherichia coli (serotype O111:B4), trypan blue dye, indomethacin, PGE₂, salmeterol xinafoate, and formoterol fumarate were purchased from Sigma (St. Louis, MO, USA). Acrylamide, SDS, Tris, HEPES, RPMI 1640 medium, phosphate-buffered saline (PBS) and bovine serum albumin (BSA) were obtained from Eurobio Biotechnology (Les Ulis, France). Roflumilast was synthesized by Nycomed GmbH (Konstanz, Germany; a gift from Dr. H. Tenor). Recombinant human GM-CSF (rhGM-CSF) was purchased from R&D Systems Europe (Lille, France). All cell culture plastics were from CML (Nemours, France). Specific antibodies against β₂-adrenoceptors and β-actin were obtained from Thermo Scientific (Vilnius, Lithuania) and Cytoskeleton (Denver, CO, USA), respectively. A Bradford protein assay and Precision Plus Protein Dual Color Standards were purchased from Bio-Rad (Hercules, CA, USA). Stock solutions of roflumilast and indomethacin were prepared in DMSO. A PGE₂ stock solution (10 mM) was prepared in ethanol. All subsequent dilutions were prepared daily in complete medium. The DMSO concentration applied to cells was 0.1%. Neither the vehicle nor any of the compounds used in this study altered cell viability. All wells were run in duplicate for each series of experiments performed with LMs or MDMs obtained from a single patient’s sample.
Isolation and culture of human LMs and MDMs
Experiments on human tissues had been approved by the regional independent ethics committee (Comité de Protection des Personnes Île de France VIII, Boulogne-Billancourt, France).

Lung tissue was obtained from 15 patients (mean ± standard error mean (SEM) age: 67 ± 4 years; gender (M:F): 10:5; FEV1/FVC ratio: 0.83 ± 0.04; 9 smokers and 6 ex-smokers; pack years: 47 ± 7) undergoing surgical resection for lung carcinoma and who had not received chemotherapy or radiotherapy. Only one donor was treated on a daily basis with a β2-adrenergic agonist. The LMs were isolated from lung parenchyma, as previously described [44]. The mean ± SEM adherent macrophage count was 191 ± 13 × 10^3 per well in 24-well plates. More than 95% of the adherent cells were macrophages, as determined by May–Grünwald–Giemsa staining and CD68 immunocytochemistry. Cell viability exceeded 90%, as assessed by trypan blue dye exclusion.

The monocytes were isolated from blood, as previously described [48]. Briefly, peripheral blood mononuclear cells from nine healthy blood donors were harvested from human buffy-coat (Etablissement Français du Sang, Ivry-sur-Seine, France) by differential centrifugation on UNI-SEP® U-10 (Novamed, Jerusalem, Israel). The experiments were performed in compliance with the French legislation on blood donation and blood product use.

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Treatment of LMs and MDMs with salmeterol and formoterol
The experiments were performed in RPMI medium supplemented with 1% FCS. The 24-well plates containing either LMs or MDMs were washed and pre-incubated with vehicle, salmeterol or formoterol for 1 h before stimulation with LPS. Following a 24 h incubation period, supernatants were collected and stored at −80 °C for later analysis of the cytokine concentration. The sub-maximal LPS concentration (10 ng.ml⁻¹) was selected on the basis of previous data [43, 44] [see Additional file 1].

To explore the LMs’ responsiveness to a β2-adrenoceptor agonist, the effect of formoterol (10 nM) was also tested in the presence (1 or 100 nM) or absence of roflumilast. Roflumilast acts as a selective PDE4 inhibitor up to a concentration of 1 μM [49]. This compound has been shown to enhance the inhibitory effect of cAMP-inducing agents (such as PGE2) on the LPS-induced release of cytokine by LMs [44]. In this series of experiments, PGE2 (10 nM) was used as an internal control for the LMs’ responsiveness to a cAMP-elevating agent [42, 44]. In order to avoid any interference of the LPS-induced production of endogenous prostanoids on the response to formoterol and PGE2, the experiments were performed in presence of indomethacin (1 μM) [44].

Measurement of cytokine production
The levels of cytokine in the supernatants were measured using the Duoset ELISA kit (R&D Systems Europe). The optical density was determined at 450 nm (MRX II, Dynex Technologies, Saint-Cloud, France). Cytokine levels were expressed in ng per 10^6 cells. The detection limits of these assays were 4 pg.ml⁻¹ for CCL3 and IL-1β, 8 pg.ml⁻¹ for TNF-α, CCL2 and CCL4, 9 pg.ml⁻¹ for IL-6, and 32 pg.ml⁻¹ for IL-8.

Expression of β2-adrenoceptors on LMs and MDMs
For real-time quantitative-PCR (RT-qPCR) analysis, LMs or MDMs (stimulated or not with LPS for 24 h) were harvested in TRIzol® reagent (Life Technologies, Saint Aubin, France). The RNA’s intactness was determined by running an aliquot of each sample on an Experion™ automated electrophoresis station (Bio-Rad, Marnes-la-Coquette, France). Next, 1 μg of total RNA was reverse-transcribed using SuperScript® III First-strand SuperMix kit (Life Technologies). Specific TaqMan® arrays based on predesigned reagents (Life Technologies) were used for the analysis of β2-adrenoceptor transcripts (ADRB2). RT-qPCR was performed using Gene Expression Master Mix (Life Technologies) with 20 ng of cDNA in a StepOnePlus thermocycler (Life Technologies). The thermal cycling conditions were as follows: initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The housekeeping genes coding for hypoxanthine phosphoribosyltransferase (HPRT1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used for signal normalization. The relative expression of mRNAs was calculated according to the 2^ΔCt method [50].

For Western blotting, LMs and MDMs were incubated with medium alone or LPS for 24 h. The cells were then washed with PBS and lysed for 15 min in an appropriate buffer (Cytobuster, Novagen, San Diego, CA, USA) containing protease inhibitor cocktail and phosphatase inhibitor cocktail (Roche, Mannheim, Germany) on ice. Equal amounts of cell lysate (30 μg) were separated on 10% SDS-PAGE gels and then transferred onto nitrocellulose membranes. The membranes were blocked for 1 h with 5% w/v non-fat powdered milk in Tris base containing 0.1% Tween 20. Next, the membranes were incubated with a mouse monoclonal antibody specific for
human β2-adrenoceptors (Thermo Scientific, Vilnius, Lithuania) and diluted (1/1000) for 2 h at room temperature. After washing, the membranes were incubated for 2 h with a horseradish-peroxidase-conjugated anti-mouse antibody (Dako, Glostrup, Denmark). The membranes were then incubated with an enhanced chemiluminescence solution for 1 min and quantified with QuantityOne 4.2.1 (Bio-Rad, Marnes-La-Coquette, France).

Statistical analysis
Data were expressed as the mean ± SEM; n represents the number of patients from whom MDM or LM preparations were obtained. Wilcoxon’s test or a one-way ANOVA for repeated measures was followed by Dunnett’s post-tests, as appropriate. The threshold for statistical significance was set to p ≤ 0.05.

Results
Effects of LPS on cytokine production by MDMs and LMs
There was no significant difference between unstimulated LMs and MDMs in terms of the production of TNF-α, IL-1β, and the chemokines (IL-8, CCL2, CCL3, and CCL4). However, IL-6 production was higher in MDMs. Following incubation with LPS, the release of all cytokines other than TNF-α and CCL4 was greater for LMs than for MDMs (Table 1).

Effects of formoterol and salmeterol on LPS-induced cytokine release by MDMs and LMs
We next investigated the effects of serial increases in the concentration (10^{-11} to 10^{-7} M) of formoterol and salmeterol on LPS-induced cytokine release. In MDMs, formoterol and salmeterol inhibited the LPS-induced production of TNF-α, IL-6 and the three CCL chemokines at concentrations greater than or equal to 10^{-10} M (Figs. 1 and 2). The respective effects of formoterol and salmeterol on the (weak) production of IL-1β were highly variable from one preparation to another. The production of IL-8 was not altered by the two LABAs. In sharp contrast to the results for MDMs, formoterol and salmeterol did not alter the LPS-induced production of any of the seven cytokines by LMs (Figs. 1 and 2). To definitively establish that the LMs’ lack of response is not restricted to these two LABAs, we performed additional experiments on four preparations of MDMs and LMs with salbutamol at 1 μM (a concentration that causes maximal relaxation of isolated human bronchus and is equipotent to the concentrations used in the present study with the LABAs (0.01 μM for formoterol and 0.1 μM for salmeterol)). Our results confirmed that this short-acting β2-adrenoceptor agonist inhibited MDMs (to much the same extent as in the work by Gill et al. [45]) but had no effect on LMs [see Additional file 2].

Effect of formoterol on LPS-induced cytokine release in the presence of roflumilast
Since formoterol was more potent than salmeterol in altering LPS-induced cytokine production by MDMs, we also looked at whether the presence of roflumilast could unmask an effect of 10^{-8} M formoterol on the LPS-induced release of TNF-α and the CCL chemokines by LMs. In this series of experiments, formoterol has no effect alone or in the presence of roflumilast on LPS-induced release of TNF-α, CCL2, CCL3 and CCL4 (Fig. 3). In contrast, the greater inhibitory effect of PGE2 on production of the four cytokines in the presence of roflumilast evidences the latter drug’s effect on a cAMP-elevator other than the LABAs in LMs (Fig. 3). In addition, formoterol did not increase significantly the inhibitory effect of PGE2 (data not shown).

Expression of β2-adrenoceptors on MDMs and LMs in presence and absence of LPS
Levels of β2-adrenoceptor transcript expression were similar in MDMs and LMs (Table 2), whereas β1- and β3-adrenoceptor transcripts were only found in macrophages from two and three patients, respectively (data not shown). Strikingly, incubation of LMs with LPS for 24 h induced an approximately 7-fold decrease in β2-adrenoceptor expression (Table 2).

To determine whether the LMs’ absence of response to the β2-adrenoceptor agonists was related to a loss of β2-adrenoceptors relative to MDMs, we performed a Western blot analysis. As shown in Fig. 4, MDMs (but not LMs) expressed β2-adrenoceptors. LPS treatment for 24 h did not alter the expression of the β2-adrenoceptors in MDMs.

Discussion
Our present results notably showed that (i) cytokine production in response to LPS differs in MDMs and
LMs, (ii) salmeterol and formoterol exert an inhibitory effect on the LPS-induced production of TNF-α, IL-6, CCL2, CCL3, and CCL4 by MDMs, (iii) the two LABAs were strikingly devoid of any effect on LMs, and (iv) Western blots revealed β2-adrenergic receptor in MDMs but not in LMs.

We confirmed the recent report in which formoterol and salmeterol can inhibit the LPS-induced release of TNF-α and IL-6 from MDMs [30]. We extended these findings to three CCL chemokines (CCL2, CCL3 and CCL4) involved in the recruitment of monocytes, immature dendritic cells and T cells [51–53]. The range of concentrations at which these two LABAs influence LPS-induced cytokine production is suggestive of a β2-adrenoceptor-dependent mechanism; this is also suggested by the attenuating effect of a β2-adrenoceptor antagonist on formoterol’s inhibitory action [30]. We also confirmed that the two LABAs did not alter the LPS-induced production of IL-1β and IL-8.

In a very recent study [45], salmeterol and indacaterol were found to inhibit the LPS-induced production of TNF-α and IL-6 by human LMs. However, four other β2-adrenoceptor agonists (formoterol, salbutamol, terbutaline and isoprenaline) were inactive, and the inhibitory effect of the two LABAs was only observed at a concentration (10⁻⁵ M) that is at least 100-fold higher than those used in the present study and caused maximum relaxation of isolated human bronchi [46, 47]. These differences in the inhibitory activities of the various β2-adrenoceptor agonists and the high concentration of the two active LABAs used in Gill et al.’s study calls into question both the clinical relevance of these results and the involvement of a β2-adrenoceptor-mediated effect. It should be noted that the inhibitory effect of indacaterol was only partly reversed by a selective β2-adrenoceptor antagonist, and the inhibitory effect of salmeterol was not reversed [45]. Moreover, the production of TXB₂ by LMs stimulated with either zymosan or the calcium ionophore A23187 was not inhibited by salbutamol (at concentrations up to 10⁻⁵ M), and the inhibitory effect of salmeterol was not blocked by propranolol - further suggesting that the effects of high concentrations of LABAs are not mediated by β2-adrenoceptors in LMs [38], as also reported for human monocytes [27, 38].
Furthermore, four β2-adrenoceptor agonists (salmeterol, formoterol, salbutamol, and terbutaline) did not alter LPS- or zymosan-induced LTB4 release from LMs at concentrations up to 10^{-5} M [39]. Taken as a whole, these results suggest that the inhibitory effects of β2-adrenoceptor agonists on LMs is weak or even null, and might only be produced at very high concentrations via a β2-adrenoceptor-independent mechanism. Given that macrophages express membrane-associated CD14, activation of the TLR4/MD-2 complex by LPS does not therefore require dimerization of the complex induced by LPS binding protein (LBP) [54]. Nevertheless, LBP (if required) was present in the FCS added to the culture medium. Hence, LBP and CD14 do not account for the results and conclusions of the present work.

MDMs differentiated by treatment with GM-CSF have been typically considered to be phenotypically and “behaviorally” similar to LMs [30, 32, 55]. However, recent high-throughput analyses have revealed remarkable differences in gene expression between MDMs and LMs; these differences include the transcripts for G-protein-coupled receptors [35, 36]. These results call into question the use of macrophage surrogates (such as MDMs) to mimic the behavior of LMs. Although β2-adrenoceptor transcript levels were similar in MDMs and LMs (35 and the present study), we found that protein levels of these receptors (as assessed by Western blotting) were much lower in LMs than in MDMs. Western blotting based on a peroxidase-conjugated secondary antibody is probably less sensitive than radioligand binding methods for detecting
receptors. Human macrophages isolated from bronchoalveolar lavages (either by elutriation or adherence to culture dishes) were found to express a moderate density of $\beta_2$-adrenoceptors in radioligand binding studies [21, 22]. In both of the latter studies [21, 22], the $\beta_2$-adrenoceptors appear to be functionally coupled to adenylate cyclase; exposure to high concentrations of isoprenaline ($\geq 10^{-7}$ M) in the presence of the PDE inhibitor isobutylmethylxanthine resulted in increased cAMP accumulation. However, the increase in cAMP was much lower in the absence of isobutylmethylxanthine, and the two studies did not determine whether the increase in cAMP levels impacted macrophage function. It is noteworthy that in a subsequent study by one of the research groups, $\beta_2$-adrenoceptor agonists did not alter the LPS- or zymosan-induced release of LTB4 from LMs [39] suggesting that the signal induced by the agonists was not strong enough to inhibit the effect of either LPS or zymosan. Furthermore, in the presence of roflumilast at a concentration that enhances the inhibitory influence of PGE2 on LPS-induced TNF-$\alpha$ and chemokine production by LMs, we found that formoterol also remained inactive. This finding suggests that stimulation of the $\beta_2$-adrenoceptors did not increase the cAMP level enough to inhibit the production of the four cytokines. In line with these results, isoprenaline alone or in combination with a PDE inhibitor had

**Table 2** Expression of $\beta_2$-adrenoreceptor mRNA transcripts (ADRB2) in human MDMs and LMs

|          | Relative expression in control (LPS-) | Relative expression after LPS exposure | Fold-change for LPS versus control |
|----------|---------------------------------------|---------------------------------------|-----------------------------------|
| MDMs     | 172.6 [134.2, 228.7]                   | 108.6 [76.2, 158.4]                   | −1.6                              |
| LMs      | 310.8 [187.1, 546.0]                   | 44.7 [23.6, 75.3]                     | −7.5                              |

The quoted result is the median [min, max] × 1000 of 3 to 5 independent experiments

**Fig. 3** Effects of formoterol, PGE2 and roflumilast on LPS-induced TNF-$\alpha$, CCL2, CCL3 and CCL4 release from LMs. Cells were pre-incubated with indomethacin (1 µM) for 30 min, followed by incubation with roflumilast (1 nM or 100 nM), PGE2 (10 nM), formoterol (10 nM) or vehicle for another 30 min prior to stimulation with LPS (10 ng.ml$^{-1}$) for 24 h. The data represent the mean±SEM of 6 different experiments, *p < 0.05, **p < 0.01, ***p < 0.001 vs. LPS + PGE2 treatment; #p < 0.05 vs. LPS

**Fig. 4** Western blot analysis of the expression of $\beta_2$-adrenoceptors on MDMs and LMs. MDMs and LMs were incubated with medium alone (control) or LPS (10 ng.ml$^{-1}$) for 24 h. Cell lysates were immunoblotted with a $\beta_2$-adrenoceptor-specific antibody.
any inhibitory effect on the release of eicosanoids induced by zymosan or IgE/anti-IgE complexes by LMs. These results for LMs contrast with the additive effects of formoterol and a β2-PDE inhibitor (roflumilast or rolipram) in human monocytes and MDMs [30, 56].

However, forskolin inhibits TXB2 release [37] and PGE2 (41, 42, 44) and the present study), NECA and roflumilast [43, 44] inhibit the LPS-induced production of cytokines by LMs - demonstrating that other cAMP elevators than LABAs are able to curb the production of eicosanoids or cytokines from LMs. Since the adenyllylcyclase/cAMP/cAMP-dependent protein kinase A axis stimulated by PGE2 reduces the LPS-induced cytokine production [42], the β2-adrenoceptor agonists’ lack of effect in LMs is probably due to the low expression of β2-adrenoceptors in these cells and thus insufficient stimulation of the pathway. The use of ten-fold lower concentrations of LPS (to markedly reduce the strength of the stimulus) unmasked a modest inhibitory effect of salbutamol on the release of TNF-α by LMs [45] - suggesting that the β2-adrenoceptor-dependent rise in cAMP content might be only sufficient to counteract a relatively weak inflammatory stimulus. Lastly, the absence of an inhibitory effect of formoterol in LMs (evidenced in the present study) rules out the involvement of this cell type in the inhibitory effect of this LABA [57] and olodaterol [14] on LPS-induced cytokine release by human lung explants. Macrophages have been implicated in the pathophysiology of COPD and (to a lesser degree) in the inflammatory load in asthma. However, given the absence of LABAs’ effects on LMs in vitro, macrophages are unlikely to account for these compounds’ anti-inflammatory effects.

Conclusion

Our present results showed that concentrations of β2-adrenoceptor agonists that cause the relaxation of isolated human bronchus can inhibit cytokine production by LPS-stimulated MDMs but not by LPS-stimulated LMs - even in the presence of a PDE inhibitor. The LMs’ lack of response could be due to low β2-adrenoceptor expression and thus an insufficiently strong cAMP-dependent trigger for the LPS-induced inflammatory response, since other cAMP elevators were able to inhibit the LPS-induced responses. The present results highlighted the lack of a clinically relevant, anti-inflammatory effect of β2-adrenoceptor agonists on LMs.

Additional files

| Additional file 1: | LPS concentration-response data for MDMs and LMs, and time-course experiments in LMs (figures). (PDF 94 kb) |
|-------------------|---------------------------------------------------------------------------------------------------------|
| Additional file 2: | Effect of salbutamol (1 μM) on LPS-induced cytokine production by MDMs and LMs (figures). (PDF 52 kb) |

Abbreviations

BSA: Bovine serum albumin; cAMP: Cyclic AMP; COPD: Chronic obstructive pulmonary disease; DMSO: Dimethyl sulfoxide; FCS: Fetal calf serum; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; HDMs: House dust mites; IL: Interleukin; LMs: Human lung macrophages; LPS: Lipopolysaccharide; LTb4: Leukotriene B4; MDMs: Human monocyte-derived macrophages; PBS: Phosphate-buffered saline; PDE4: Phosphodiesterase 4; rhGM-CSF: Recombinant human GM-CSF; TLR4: Toll-like receptor 4; TNF: Tumor necrosis factor; TXB2: Thromboxane B2.

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none.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

EN, SG-D, MB, LSCP, TV and CA performed the research. VL and PD designed the research. TV, SG-D, MB and PD analyzed the data. TV and PD wrote the paper. All authors read and approved the final manuscript.

Competing interests

The authors have no conflicts of interest with regard to the present study, which was not sponsored by a company. PD and EN have received research funding from Boehringer-Ingelheim in the field of respiratory research. PD has received consulting fees, honoraria for lectures and/or participation in scientific advisory boards from AstraZeneca, Boehringer Ingelheim, Chiesi, GlaxoSmithKline and Novartis. TV, CA, MB, VL, SG-D, HS and LP have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The use of human lung tissue for in vitro experiments was approved by the local independent ethics committee (Comité de Protection des Personnes Ile de France VIII, Boulogne-Billancourt, France). In line with the French legislation on clinical research (and approved by the independent ethics committee), only verbal informed consent (rather than written consent) was required. A brief note from the investigating physician (stating that consent had been requested and verbally granted at the time of the consultation) was included in the patient’s medical records. If the patients disagreed (by ticking a box and signing the information sheet [available on request]), their lung tissue (if any) was not made available to our laboratory.

Hence, informed consent was obtained from each patient.

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43. Buenestado A, Grassin Delyle S, Arnould I, Bensard F, Naline E, Blouquit-Laye S, Chapelier A, Bellamy JF, Devillier P. The role of adenosine receptors in regulating production of tumour necrosis factor-alpha and chemokines by human lung macrophages. Br J Pharmacol. 2010;159:1304–11.

44. Buenestado A, Grassin-Delyle S, Guitard F, Naline E, Faisy C, Israel-Biet D, Sage E, Bellamy JF, Tenor H, Devillier P. Roflumilast inhibits the release of chemokines and TNF-alpha from human lung macrophages stimulated with lipopolysaccharide. Br J Pharmacol. 2012;165:1877–90.

45. Gill SK, Marriott HM, Suvarna SK, Peachell PT. Evaluation of the anti-inflammatory effects of beta-adrenoceptor agonists on human lung macrophages. Eur J Pharmacol. 2016;793:49–55.

46. Bouyssou T, Casarosa P, Naline E, Pestel S, Konetzki I, Devillier P, Schnapp A. Pharmacological characterization of olodaterol, a novel inhaled beta2-adrenoceptor agonist exerting a 24-hour-long duration of action in preclinical models. J Pharmacol Exp Ther. 2010;334:53–62.

47. Naline E, Trifileff A, Fairhurst RA, Advenier C, Molimard M. Effect of indacaterol, a novel long-acting beta2-agonist, on isolated human bronchi. Eur Respir J. 2007;29:575–81.

48. Gicquel T, Victoni T, Fautrel A, Robert S, Génelec F, Guézinger M, Couillin I, Catros V, Boichot E, Lagente V. Involvement of purinergic receptors and NOD-like receptor-family protein 3-inflammasome pathway in the adenosine triphosphate-induced cytokine release from macrophages. Clin Exp Pharmacol Physiol. 2014;41:279–86.

49. Hatzelmann A, Schubt C. Anti-inflammatory and immunomodulatory potential of the novel PDE4 inhibitor roflumilast in vitro. J Pharmacol Exp Ther. 2001;297:267–79.

50. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001;25:402–8.

51. Bachelerie F, Ben-Baruch A, Burkhardt AM, Combadiere C, Farber JM, Graham GJ, Horuk R, Sparse-Ulrich AH, Locati M, Luster AD, et al. International Union of Basic and Clinical Pharmacology. [corrected]. LXXXIX. Update on the extended family of chemokine receptors and introducing a new nomenclature for atypical chemokine receptors. Pharmacol Rev. 2014;66:1–79.

52. Brusselle GG, Joos GF, Bracke KR. New insights into the immunology of chronic obstructive pulmonary disease. Lancet. 2011;378:1015–26.

53. Ravi AK, Khurana S, Lemon J, Plumb G, Healy L, Catley M, Vestbo J, Singh D. Increased levels of soluble interleukin-6 receptor and CCL3 in COPD sputum. Respir Res. 2014;15:103.

54. Tsukamoto H, Fukudome K, Takao S, Taneyaoshii N, Kimoto M. Lipopolysaccharide-binding protein-mediated Toll-like receptor 4 dimerization enables rapid signal transduction against lipopolysaccharide stimulation on membrane-associated CD14-expressing cells. Int Immunol. 2010;22:271–80.

55. Komuro I, Keicho N, Imamoto A, Akagawa KS. Human alveolar macrophages and granulocyte-macrophage colony-stimulating factor-induced monocyte-derived macrophages are resistant to H2O2 via their high basal and inducible levels of catalase activity. J Biol Chem. 2001;276:24360–4.

56. Tannheimer SL, Soensenn EA, Harar AC, Mansfield CM, Wright CD, Salmon M. Additive anti-inflammatory effects of beta 2 adrenoceptor agonists or glucocorticosteroid with roflumilast in human peripheral blood mononuclear cells. Pulm Pharmacol Ther. 2012;25:178–84.

57. Buenestado A, Chaumais MC, Grassin-Delyle S, Risse PA, Naline E, Longchamp E, Tenor H, Devillier P. Roflumilast inhibits lipopolysaccharide-induced tumor necrosis factor-alpha and chemokine production by human lung parenchyma. PloS One. 2013;8:e74640.