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Effects of formoterol and budesonide on GM-CSF and IL-8 secretion by triggered human bronchial epithelial cells

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ABSTRACT: The effect of formoterol, alone and in combination with budesonide, upon tumour necrosis factor-α stimulated (10 ng·mL^{-1}) human bronchial epithelial cells was investigated.

Addition of formoterol ($\geq 10^{-10}$ M) reduced granulocyte macrophage-colony stimulating factor (GM-CSF) levels, as assessed by enzyme-linked immunosorbent assay, by 40–50% and increased interleukin (IL)-8 levels by ~50%. The effects of formoterol were long lasting (23 h). Budesonide (10^{-8} M) reduced the amounts of both cytokines (GM-CSF and IL-8) by 40%. Simultaneous addition of formoterol and budesonide reduced GM-CSF levels ~75%, while IL-8 levels were decreased ~40%, similar to the reduction obtained with budesonide alone. The glucocorticoid receptor (GR) antagonist RU486 did not influence the effect of formoterol, suggesting no involvement of the GR. Formoterol rapidly induced an elevation in intracellular cyclic adenosine monophosphate, which was reduced in the presence of propranolol. In addition, the alterations in cytokine secretion induced by formoterol could be fully blocked by propranolol, demonstrating that these effects are β_2-receptor mediated.

In conclusion, the combination of budesonide and formoterol reduces the secretion of granulocyte macrophage-colony stimulating factor to basal levels and counteracts the capacity of formoterol alone to induce interleukin-8 production, modulations which may facilitate improved asthma control.

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Recent clinical studies have demonstrated that the combination of inhaled short- or long-acting β_2-agonists with inhaled glucocorticoids results in better asthma control than higher doses of glucocorticoids alone [1–3]. These clinical findings have been complemented with *in vitro* mechanistic studies, in which β_2-agonists (or other cyclic adenosine monophosphate (cAMP) elevating agents) and glucocorticoids were demonstrated to have additive effects on the inhibition of cytokine release and adhesion molecule expression [4, 5].

The mechanism(s) by which β_2-agonists exert their anti-inflammatory effects is not yet fully understood. One possibility may be through the direct interaction of the transcription factor cAMP responsive element binding (CREB) protein with proinflammatory transcription factors, such as activating protein-1 (AP-1) and nuclear factor-κB (NF-κB). Transcription factor interaction is a major mechanism by which glucocorticoids repress inflammation, since most genes that are repressed by glucocorticoids do not contain glucocorticoid responsive elements (GRE) in their promoters and are thus not able to be transcriptionally activated by glucocorticoids [6]. Another pathway through which β_2-agonists might repress inflammation is *via* activation of the glucocorticoid receptor (GR). It is known that the activation of protein kinases A and C (PKA and PKC) stimulates GR activities by phosphorylation of specific sites at the N-terminus side of the GR [7]. Recently, it was shown, in primary pulmonary fibroblasts and vascular smooth muscle cells, that β_2-agonists can activate the GR, resulting in nuclear translocation, deoxyribonucleic acid (DNA)-binding and initiation of transcription of a GRE-regulated reporter gene. If airway target cells employ this mechanism, it could in part explain the anti-inflammatory properties of β_2-agonists.

There is increased production of several cytokines, *i.e.* granulocyte macrophage-colony stimulating factor (GM-CSF), interleukin (IL)-5, IL-6, and IL-8, in airways of patients with asthma. Airway epithelial cells may contribute considerably to the establishment and/or maintenance of airway inflammation by the production of these cytokines. Bronchial epithelial cells express GR and β_2-receptors [9, 10], which may mediate parts of the described anti-inflammatory actions of glucocorticoids and β_2-agonists [5, 11–15]. Thus, bronchial epithelial cells are able to both maintain airway inflammation and respond to therapeutic agents commonly used in asthma therapy.

The present study was performed to investigate whether the long-acting β_2-agonist formoterol modulates cytokine release by triggered epithelial cells and, if so, whether this is mediated through the GR. The

potential interaction of formoterol with the cytokine blocking effects of the glucocorticoid budesonide was also studied. The specificity of the formoterol-mediated effects was studied by blocking β_2-receptors with the β-antagonist propranolol and by measuring cAMP levels in the cells. Adding the GR-antagonist RU486 elucidated the extent of involvement of the GR.

Materials and methods

Cells and culture medium

Primary normal human bronchial epithelial cells (Clonetics, NHBE 4892) obtained from Cytotech ApS (Copenhagen, Denmark) were cultured in bronchial epithelial cell growth medium (BEGM) consisting of 500 mL bronchial epithelial basal medium (BEBM) complemented with 52 $\mu g \cdot mL^{-1}$ bovine pituitary extract (BPE), 0.5 $\mu g \cdot mL^{-1}$ hydrocortisone, 0.5 $ng \cdot mL^{-1}$ human epithelial growth factor (hEGF), 0.5 $\mu g \cdot mL^{-1}$ epinephrine, 10 $\mu g \cdot mL^{-1}$ transferrin, 5 $\mu g \cdot mL^{-1}$ insulin, 0.1 $ng \cdot mL^{-1}$ retinoic acid, 50 $\mu g \cdot mL^{-1}$ GA-1000 (Cytotech Aps), and 6.5 $ng \cdot mL^{-1}$ triiodothyronine (T3) (Cytotech ApS). During the experiments, the cells were grown on incomplete BEGM, lacking hydrocortisone, retinoic acid and epinephrine, hereafter referred to as -BEGM.

Cell culture

Cells were grown to ~60% confluence in uncoated 24-well plates (Costar, Cambridge, MA, USA). Experiments were started by culturing the cells in -BEGM for 24 h after which time 10 $ng \cdot mL^{-1}$ human recombinant tumour necrosis factor-α (TNF-α) (Genzyme/Novakemi AB, Enskede, Sweden) was added in the presence or absence of racemic (50/50) formoterol (AstraZeneca, Lund, Sweden) and/or racemic (50/50) budesonide (AstraZeneca). Cells were then cultured for an additional 24 h, along with appropriate vehicle controls. To assess whether the effect of formoterol was through the β_2-receptor, the β_2-antagonist propranolol (ICI, Macclesfield, UK) was used. To assess the activities of formoterol in the presence of GR-antagonists, RU486 (Sigma, Stockholm, Sweden) was employed. After 24 h, when cells were at 80–90% confluence, the experiments were ended by centrifuging the plates for 10 min at $208 \times g$, 4°C. The supernatant was frozen (-70°C) and the DNA was quantitated in each well. Each experiment was performed in duplicate and supernatents from these duplicates were pooled prior to freezing. Each experiment was repeated at least three times with different batches of normal human bronchial epithelial (NHBE) 4892 cells at different passage numbers.

Deoxyribonucleic acid measurement

DNA was quantitated according to the previously described method from Blaheta *et al.* [16], with some alterations. After centrifuging the 24-well plates and removing the supernatant as described above, the plates were kept on ice. Six-hundred μL phosphate buffered saline or aliquots of double stranded DNA to generate a standard curve, were added to the wells, followed by 400 μL 10 $\mu g \cdot mL^{-1}$ H33342. The plates were covered with foil and incubated for 30 min at room temperature. The fluorescence was measured and DNA amounts were calculated in $\mu g \cdot mL^{-1}$.

Cytokine assays

GM-CSF and IL-8 levels were determined for each experimental condition with enzyme-linked immunosorbent assay (ELISA) kits from R&D Systems (Abingdon, UK), according to the manufacturer's instructions. IL-8 samples were diluted 10-fold before measuring, while GM-CSF was measured neat. The levels of GM-CSF and IL-8 were determined in $pg \cdot mL^{-1}$ culture medium, and related to the DNA content per well.

Cyclic adenosine monophosphate assay

cAMP levels were determined at different times (5–30 min) subsequent to the addition of formoterol at different concentrations (10^{-9}–10^{-7} M), with a two stage scintillation proximity assay (SPA) from Amersham (Amersham Pharmacia Biotech, Buckinghamshire, UK), according to the manufacturer's protocol.

Statistics

Experiments were repeated at least three times. All cytokine data are expressed as mean±SD for all studies performed. Comparisons between absolute values ($pg \cdot \mu g^{-1}$ DNA) of the cytokines were made using analysis of variance (ANOVA). The cAMP data was represented as individual experiments, and the statistical analysis was performed with unpaired t-tests. Data were considered to be significant when $p \leq 0.05$.

Results

Effect of formoterol on granulocyte macrophage-colony stimulating factor and interleukin-8 levels

Exposure of cells to 10 $ng \cdot mL^{-1}$ TNF-α resulted in a 5–6-fold increase in secreted GM-CSF and IL-8 levels. GM-CSF levels rose from ~12 $pg \cdot \mu g^{-1}$ DNA under basal conditions to 63 $pg \cdot \mu g^{-1}$ DNA upon stimulation with TNF-α. Similarly, IL-8 levels increased from ~128 $pg \cdot \mu g^{-1}$ DNA to 689 $pg \cdot \mu g^{-1}$ DNA. Formoterol modulated GM-CSF and IL-8 levels in opposite manners. GM-CSF was dose-dependently reduced (fig. 1a) to 60% of the TNF-α stimulated value, with a threshold level for significance at 10^{-10} M. IL-8 amounts were enhanced by formoterol (fig. 1b), to a maximum level of 160% of

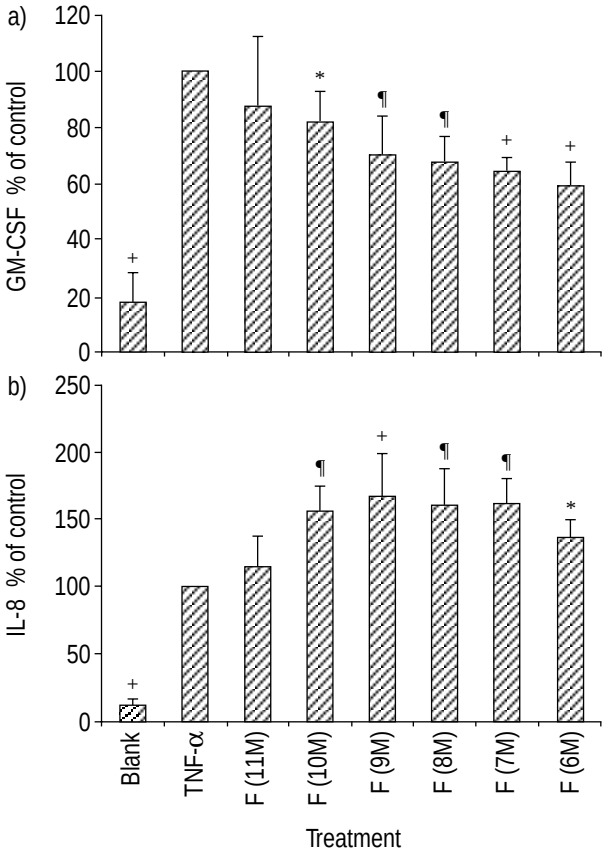

Fig. 1. – a) Granulocyte macrophage-colony stimulating factor (GM-CSF) and b) interleukin (IL)-8 levels in bronchial epithelial cells exposed for 24 h to 10^{-11} – 10^{-6} M (11M – 6M) formoterol (F). Tumour necrosis factor-α (TNF-α) (10 ng·mL^{-1}) stimulated GM-CSF levels were decreased by F at concentrations of 10^{-10} M and higher. TNF-α-induced IL-8 levels were increased with a similar threshold level. Significant differences *versus* the TNF-α control: *: $p<0.05$; ¶: $p<0.005$; $^{+}$: $p<0.001$.

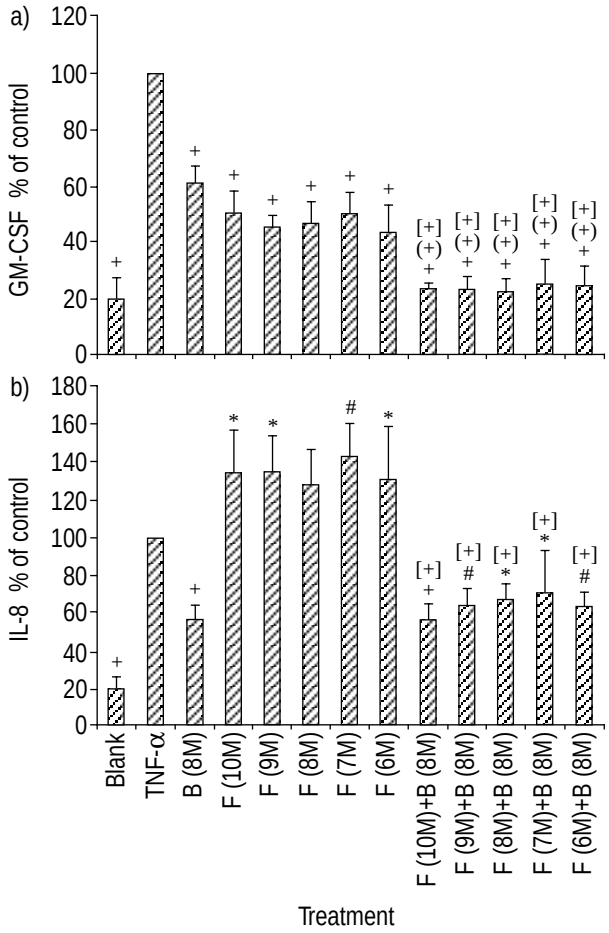

Fig. 2. – Effects of 10^{-8} M (8M) budesonide (B) and 10^{-10} – 10^{-6} M (10M – 6M) formoterol (F) added simultaneously or separately for 24 h to bronchial epithelial cells. a) The granulocyte macrophage-colony stimulating factor (GM-CSF) levels increased by 10 ng·mL^{-1} tumour necrosis factor-α (TNF-α), were decreased by B and F, and further decreased to basal levels when B and F were added together. b) Interleukin (IL)-8 levels decreased by B were not influenced by F, even at a 100-fold F excess. Significant differences *versus* TNF-α, (budesonide alone) and [the respective formoterol concentration alone]. Symbols representing p-values are *: <0.05; $^{\#}$: <0.01; $^{+}$: <0.001.

that secreted by TNF-α stimulated cells, with an equally low threshold level.

Pulse experiments were performed to better replicate the shorter exposure duration upon therapeutic inhalation. Cells were exposed for 1 h to TNF-α and formoterol, washed twice, and continued growing for another 23 h in a medium containing only TNF-α [17]. GM-CSF and IL-8 levels were regulated similarly as described in figure 1. However, an ~100-times higher threshold level of formoterol was required to inhibit GM-CSF and to enhance IL-8 production, respectively.

Interaction between formoterol and budesonide

Cells were incubated with TNF-α in the presence of both formoterol (10^{-10} – 10^{-6} M) and budesonide (10^{-8} M) for 24 h, and the effects were compared to that of cells exposed to TNF-α and either drug alone. The budesonide concentration was selected as being on the intermediate part of its dose-response curve and as being a concentration documented in lung tissue of humans receiving an inhaled dose of this compound [18]. Figure 2 demonstrates that the reduction of GM-CSF induced separately by budesonide (40%) or formoterol (50 – 55%) was markedly enhanced to 75% when the compounds were added in combination. A more surprising finding was seen for IL-8. Budesonide 10^{-8} M alone inhibited IL-8 secretion by 45%, whereas formoterol (10^{-10} – 10^{-6} M) alone increased the secretion by 35% (fig. 2). However, when added together, the inhibition by budesonide remained unchanged, even when a 100-times higher concentration of formoterol than budesonide was added (fig. 2). Similar results for both GM-CSF and IL-8 were obtained with two other β_2-agonists (salmeterol and terbutaline) in combination with budesonide (fig. 3). However, it appears as though the decreased levels of IL-8 elicited by budesonide were more counteracted by salmeterol and terbutaline than by formoterol.

The interaction experiments were partly repeated

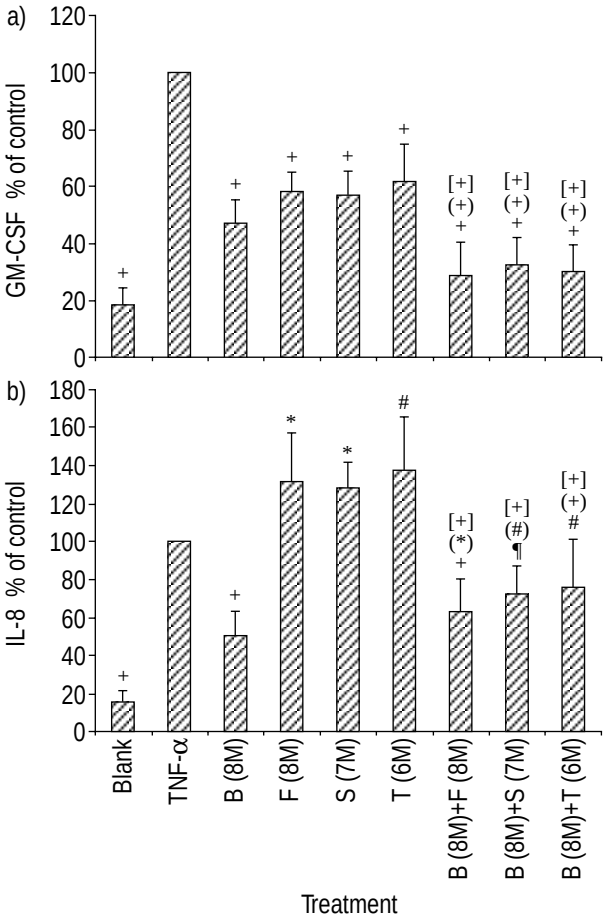

Fig. 3. – Effects of 10^{-6} M (6M) terbutaline (T), 10^{-7} M (7M) salmeterol (S), and 10^{-8} M (8M) formoterol (F) on granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin (IL)-8 levels decreased by 10^{-8} M (8M) budesonide (B). The tumour necrosis factor-α (TNF-α) (10 ng·mL^{-1})-induced GM-CSF levels were additively reduced with all three β_2-agonists (S, F and B). The stimulated IL-8 levels were still lowered by B, despite the addition of a β_2-agonist. Significant differences *versus* TNF-α, (B), and [the respective β_2-agonist alone]. Symbols representing p-values are *: <0.05; $^{\#}$: <0.01; ¶: <0.005; and $^{+}$: <0.001.

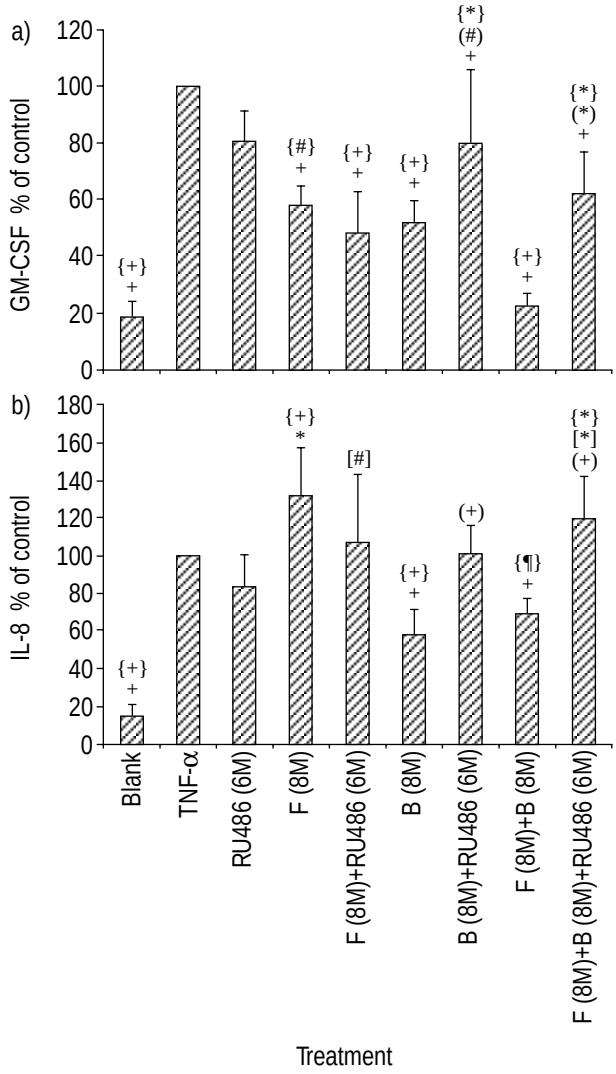

Fig. 4. – The effect of RU486, a glucocorticoid receptor antagonist, on formoterol (F) and budesonide (B) activity. Addition of 10^{-6} M (6M) RU486 to cells exposed to 10^{-8} M (8M) B prevented the reduction of both a) granulocyte macrophage-colony stimulating factor (GM-CSF) and b) interleukin (IL)-8. The effect of F on cytokine secretion persisted, despite the addition of RU486. Significant differences *versus* tumour necrosis factor-α (TNF-α) (10 ng·mL^{-1}), (B alone), [F alone] and {RU486 alone}. Symbols representing p-values are *: <0.05; $^{\#}$: <0.01; ¶: <0.005; and $^{+}$: <0.001.

with a 1-h pulse of both budesonide and formoterol (data not shown). The clear additive effect of formoterol and budesonide on GM-CSF levels, as seen with the continuous exposure in figure 2, was lost with this pulse approach (data not shown), but a very consistent reduction to 45% of control was observed. IL-8 levels were lowered to 90% of control in the cells exposed to the combination, being not significantly different from the 100% control value containing only TNF-α (data not shown).

Effect of RU486

The addition of 10^{-6} M RU486 did not block the formoterol (10^{-8} M) induced GM-CSF decrease (fig. 4). Since RU486 itself had some inhibitory effect on GM-CSF, a slightly enhanced reduction of GM-CSF levels was observed when adding both formoterol and RU486. As anticipated, the activity of budesonide (10^{-8} M) was fully abolished by RU486, compared to the RU486 control. Formoterol and budesonide together lowered GM-CSF levels to unstimulated levels. This was reversed by RU486 to the level seen with only formoterol (fig. 4). RU486 changed the effects of formoterol and budesonide on IL-8 secretion in a principally similar way as for the GM-CSF production, even though these interactions appeared less distinct due to its own capacity to diminish IL-8 secretion. Thus, RU486 fully abrogated the inhibiting activity of budesonide (fig. 4), but marginally counteracted the enhancement of IL-8 secretion by formoterol (when compared with the RU486 control). The combined inhibition of IL-8 secretion by formoterol and budesonide was reversed

by RU486, resulting in an enhanced secretion similar to that seen with formoterol alone.

Influence of propranolol

Formoterol increased cellular cAMP production very rapidly, with an optimal induction with 10^{-7} M formoterol after 10 min (data not shown). The increase in cAMP induced by 10^{-7} M formoterol was dose-dependently (10^{-9} – 10^{-7} M) blocked by propranolol (fig. 5), demonstrating involvement of the β_2-receptor.

To determine whether the cytokine modulating effect of formoterol was β_2-receptor mediated, cells were exposed simultaneously to formoterol and propranolol for 24 h. In figure 6, it is demonstrated that the decrease in GM-CSF secretion by 10^{-9} M formoterol involves the β_2-receptor, since it was dose-dependently inhibited by propranolol ($\geq 10^{-8}$ M). Similar effects were observed for IL-8 (fig. 6), in which case propranolol dose-dependently ($>10^{-8}$ M) inhibited the formoterol induced increase in the secretion of this cytokine.

Discussion

Recent clinical studies, in which asthma therapy using a combination of inhaled steroids plus long-acting β_2-agonists was compared to therapies employing each agent alone, show an improved therapeutic outcome on objective as well as on subjective parameters [1–3]. Two major reasons to explain this positive interaction have been proposed: either that the β_2-agonist potentiates the steroid anti-inflammatory efficacy, or that the β-agonists exert anti-inflammatory

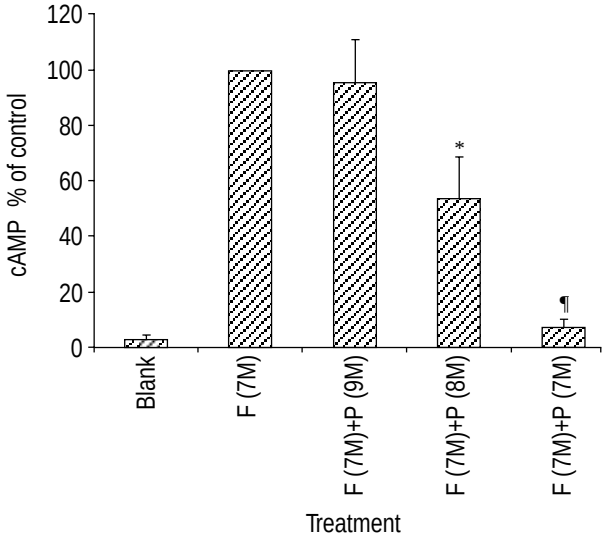

Fig. 5. – Cyclic adenosine monophosphate (cAMP) levels increased within 10 min after exposing bronchial epithelial cells to 10^{-7} M formoterol (F). This increase was blocked by the addition of propranolol (P), demonstrating involvement of the β_2-receptor. Significant differences *versus* the formoterol control: *: $p<0.05$; ¶: $p<0.005$.

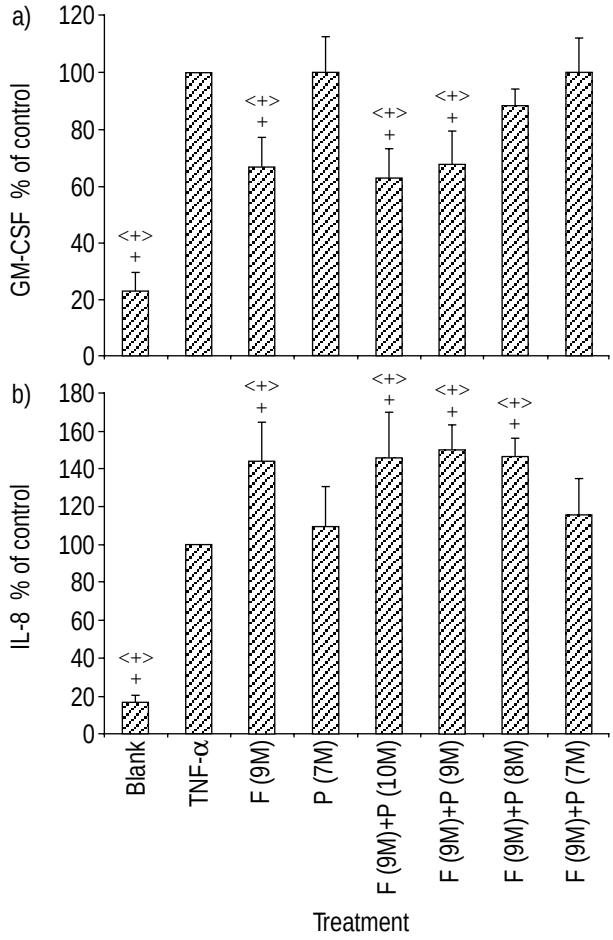

Fig. 6. – Effects of 10^{-7} – 10^{-10} M (7M – 10M) propranolol (P) on 10^{-9} M (9M) formoterol (F)-altered a) granulocyte macrophage-colony stimulating factor (GM-CSF) and b) interleukin (IL)-8 levels. Increasing the concentrations of P blunted the GM-CSF reduction by F. Also, the increased IL-8 levels were normalized by P. Significant differences *versus* tumour necrosis factor-α (TNF-α) (10 ng·mL^{-1}); and <propranolol alone>. $^{+}$: <0.001.

activity on their own [3]. It is clear that β_2-agonists, through elevations in cellular cAMP, can reduce recruitment and activity of some types of proinflammatory cells. Lavage/biopsy studies have documented that formoterol inhalation reduces the number of mast cells and eosinophils in the airways of asthmatics [19], and that salmeterol can decrease the neutrophil number [20]. These sustained *in vivo* effects exclude the possibility that there is a complete tachyphylaxis for this type of anti-inflammatory activity.

Since bronchial epithelial cells appear to be of significant importance in asthma pathophysiology, the interaction of budesonide and formoterol in an *in vitro* bronchial epithelial cell system was studied herein. A reduced exacerbation frequency was one of the major findings of studies combining inhaled steroid with long-acting β_2-agonists [2, 21]. As asthma exacerbations may be initiated at the epithelial level by secretion of chemokines and growth factors, it was the study's objective to investigate some of these aspects *in vitro*. The primary human epithelial cells used in this study secreted cytokines upon stimulation

with TNF-α, and they were sensitive to both glucocorticoids and β_2-agonists. Receptor specificity of the drug actions was tested by adding the antagonists RU486 and propranolol. GM-CSF and IL-8 were chosen for analysis, as both are important cytokines for proliferation and recruitment of cells in airway inflammation. These cytokines are of additional interest as both glucocorticoids and β_2-agonists modulate their secretion differently; budesonide inhibits the production of both, while formoterol reduces GM-CSF but enhances IL-8 production. Relevant drug concentrations were chosen to mimic the *in vivo* situation at the airway level (10^{-8} M budesonide and $10^{-10}-10^{-6}$ M formoterol), based on human kinetic studies [22], *in vitro* pharmacology [23], and the ~10 to 1 dose ratio routinely employed in these therapeutic inhalation regimens.

As observed in *in vitro* studies [5, 15], β_2-receptor agonists reduce TNF-α stimulated GM-CSF production. The high potency of formoterol (threshold concentration 10^{-10} M) depends on its strong lipophilicity, resulting in accumulation of the drug into the cell membrane during a 24-h experiment [24]. The cell membrane is the target for β_2-agonist action, as the β_2-receptors are located there, but there are few studies describing how the drug concentration evolves in the membrane over time. Since the bulk of inhaled drugs stay within the lungs for a brief period of time, the experimental setting with a 24-h incubation was complemented with a pulse exposure. In this setting, cells were exposed to formoterol for 1 h, washed, and continued growing for the remaining 23 h. The threshold level for efficacy was then 100 times higher, *i.e.* 10^{-8} M for GM-CSF when compared to the continuous exposure, but otherwise the qualitative actions of formoterol were identical. These results indicate that a formoterol pulse has a long duration of action.

It is more difficult to extrapolate an anti-inflammatory efficacy from the IL-8 enhancing property of formoterol, seen in the present authors' studies, as well as reported previously with other β_2-agonists [25]. Even though formoterol altered the GM-CSF and IL-8 production in opposite manners, it had the same very low threshold concentration (10^{-10} M) to elicit both effects. In the 1-h pulse experiment, a 100 times higher threshold was needed, but again the efficacy of the pulse lasted for 23 h. Adding propranolol confirmed that cytokine modulations (as well as the cAMP induction) were β_2-receptor mediated (the nonselective β-receptor antagonist propranolol could be used here, as bronchial epithelial cells lack β_1-receptors [9]).

Combining budesonide with formoterol resulted in a nearly full blunting of GM-CSF secretion down to basal levels. A similar improvement was obtained when budesonide was combined with salmeterol or terbutaline instead of formoterol, indicating a principally similar effect of both long- and short-acting β_2-agonists. This is interesting considering the recent concerns regarding the regular use of short-acting β_2-agonists, proposed to have negative effects on asthma control under certain conditions. However, for reducing GM-CSF, the effects of budesonide and the β_2-agonist are largely additive. This is in concordance with recent results of studies performed using human lung fibroblasts, in which budesonide and formoterol strongly blunted the IL-1β induced secretion of GM-CSF and intracellular adhesion molecule-1 (ICAM-1) [26]. In the pulse experiments, the combination of budesonide and formoterol had the preference of giving a GM-CSF block with less variance, but there was less strong support for an additive efficacy. However, the optimal *in vitro* pulsing length and interval for best concordance with airway tissue on a once or twice daily inhalation regimen are not yet known.

While budesonide and formoterol by themselves modulated TNF-α induced IL-8 production in different manners, combined continuous treatment nearly fully retained the blocking efficacy of the steroid. In the pulse experiments with generally weaker effects of the individual components, the combined treatment at least counteracted the inducing effect of formoterol. How the continuous budesonide treatment is able to counteract even high formoterol concentrations is unknown. One possibility may be that the GR binds up central transcription factors activated by TNF-α and formoterol (*i.e.* AP-1, NF-κB and CREB), preventing their binding to CREB binding protein (CBP) and/or DNA, and thereby inhibit the initiation of IL-8 gene transcription. Another option is that budesonide shortens the half-life of IL-8 messenger ribonucleic acid (mRNA), as has been reported for human lung fibroblasts exposed to dexamethasone [11].

In an article from PANG and KNOX [27], interactions between β_2-agonists and glucocorticoids on IL-8 secretion were studied in airway smooth muscle cells. Salbutamol or salmeterol only weakly enhanced the high IL-8 levels induced by TNF-α. While dexamethasone was able to reduce the TNF-α induced IL-8 levels, addition of dexamethasone to TNF-α and salbutamol/salmeterol exposed cells resulted in an enhanced reduction of IL-8 levels. Parts of these observations are in contrast with the present findings. This might be explained by the fact that IL-8 levels, after TNF-α stimulation, were ~50 times higher in the smooth muscle cells compared to the levels observed in the present study's bronchial epithelial cells. Possibly, an additional increase in IL-8 elicited by adding β_2-agonists might be difficult to achieve higher up on the response curve. Furthermore, the smooth muscle cells were preincubated with dexamethasone and salbutamol/salmeterol, in contrast to the simultaneous addition of drugs and TNF-α in the present experiments.

It has recently been suggested that the anti-inflammatory properties of β_2-agonists might be partially mediated through activation of the GR [8]. PKA, a cAMP-dependent kinase activated by β_2-agonists, stimulates GR-activity [7]. Indeed, in the article by EICKELBERG *et al.* [8], inhibition of PKA blocked GR-activation. In this manuscript, the hypothesis was tested with RU486, a GR-antagonist. No alteration of formoterol activity on GM-CSF and IL-8 was observed upon the addition of RU486. On the other hand, the anti-inflammatory activity of budesonide was totally inhibited by RU486, indicating

that RU486 had antagonistic properties on the GR under the conditions employed. The fact that formoterol was still able to reduce GM-CSF levels despite the presence of a 100-fold excess of RU486, indicates that this anti-inflammatory activity is not GR-mediated. When adding formoterol and budesonide together with RU486 the effect of the budesonide was abrogated and only the effect of formoterol remained. The formoterol induced IL-8 secretion was also not blocked by RU486. Further studies are required to elucidate whether the different results obtained here and by EICKELBERG *et al*. [8] depend on the various analytical methods and cell types used, or that the β_2-mediated translocation of GR does not lead to functional activity upon normal gene transcription, nor to binding of other transcription factors.

In conclusion, formoterol has a very low threshold concentration for reducing the granulocyte macrophage-colony stimulating factor production of cultured human bronchial epithelial cells, with a blocking efficacy of $\sim$40%. Formoterol alone enhances tumour necrosis factor-α induced interleukin-8 production by this cell type. Both of these effects are mediated through the β_2-receptor into a signal transduction pathway that does not involve the glucocorticoid receptor. Combining formoterol with budesonide leads to an improved antiasthmatic profile, as the inhibition of granulocyte macrophage-colony stimulating factor secretion results in near basal levels, and the capacity of formoterol to induce interleukin-8 production is counteracted. That the effects of formoterol are not directly mediated through the glucocorticoid receptor does not exclude interactions between glucocorticosteroids and β_2-agonist at other levels. Further *in vivo* studies, in which the airway expression/production of these cytokines is examined after combined therapy, are required in order to reveal the relevance of these cellular findings, and to determine whether such *in vivo* effects may explain the reduced exacerbation rate seen in the Formoterol and Corticosteroids Establishing Therapy study [2]. The molecular basis of these effects also remains to be elucidated in order to better predict when steroids and β_2-agonists may have a therapeutically positive or a negative interaction.

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