



Review

Molecular and cellular mechanisms underlying the therapeutic effects of budesonide in asthma



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ABSTRACT

Inhaled glucocorticoids are the mainstay of asthma treatment. Indeed, such therapeutic agents effectively interfere with many pathogenic circuits underpinning asthma. Among these drugs, during the last decades budesonide has been probably the most used molecule in both experimental studies and clinical practice. Therefore, a large body of evidence clearly shows that budesonide, either alone or in combination with long-acting bronchodilators, provides a successful control of asthma in many patients ranging throughout the overall spectrum of disease severity. These excellent therapeutic properties of budesonide basically depend on its molecular mechanisms of action, capable of inhibiting within the airways the activity of multiple immune-inflammatory and structural cells involved in asthma pathobiology.

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1. Introduction

Asthma is a phenotypically heterogeneous disorder of the airways, characterized by inflammatory and structural changes which are responsible for bronchial hyperresponsiveness and usually

reversible airflow limitation [1,2]. This chronic respiratory disease affects over 300 million asthmatic people worldwide, who will probably become more than 400 million by 2020 [3,4]. A huge array of cytokines, chemokines and growth factors, released by both immune-inflammatory and airway structural cells, significantly contribute to the development and progression of asthma [5,6]. These and other pro-inflammatory mediators maintain and amplify intercellular communications connecting T lymphocytes, mast cells, eosinophils and neutrophils, as well as bronchial epithelial cells, sub-epithelial fibroblasts and airway smooth muscle cells.

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In the so-called “T-helper (Th)2 high” or “type 2” molecular phenotypes of eosinophilic asthma, which represent the most frequent disease variants, complex pathobiologic circuits are sustained by immunologic cascades mainly orchestrated by Th2 lymphocytes, which release interleukins (IL)-4, 5 and 13 [7]. Th2 lymphocytes differentiate and expand as a consequence of a functional impairment of T regulatory (Treg) cells, which causes the activation of dendritic cells directing Th2 polarization [8,9]. Airway eosinophilia is not only driven by adaptive immune mechanisms, but can also arise from relevant innate immune responses, which depend on cellular networks involving bronchial epithelial cells and type 2 innate lymphoid cells [2,10–12].

In asthma, chronic inflammation is often associated with structural changes involving all layers of the airway wall, including epithelial shedding, hyperplasia of goblet cells and mucous glands, as well as enhanced deposition of extracellular matrix proteins, mainly produced by mesenchymal cells such as proliferating lung fibroblasts/myofibroblasts [13,14]. Moreover, a remarkable role in this process of airway remodelling is also played by enhanced angiogenesis and proliferation of airway smooth muscle cells, which together with sub-epithelial fibrosis significantly contribute to thicken bronchial walls [15,16]. An increased thickness of the epithelial reticular basement membrane appears to be closely coupled with eosinophilic airway inflammation [17]. Furthermore, the enlarged mass of airway smooth muscle detectable in many patients with asthma, seems to be directly related to disease duration and severity [18]. Overall, bronchial remodelling reduces airway lumen and leads to a progressive worsening of lung function.

Within the complex pathophysiologic context of asthma, glucocorticoids (GCs) act as the cornerstone of pharmacological treatment. Mainly administered via the inhaled route, these drugs are the most important therapeutic agents for such a widespread respiratory disease because they are capable of effectively targeting many cellular elements implicated in airway inflammation and remodelling [19,20]. Among inhaled GCs, budesonide is characterized by excellent pharmacological properties and therapeutic efficacy [21], thereby probably representing during the last decades the most used topical agent for asthma treatment, with regard to both experimental studies and clinical practice. Therefore, within the context of anti-asthma therapies, budesonide is a very reliable treatment paradigm, and its differences in clinical use with respect to the other inhaled GCs are summarized in Table 1 [22].

On the basis of the above considerations, the aim of this review article is to focus on the molecular mechanisms of action of budesonide, as well as on its main airway cellular targets involved in asthma pathobiology.

2. Budesonide: molecular mechanisms of action

All GCs, also including budesonide, are liposoluble molecules

Table 1
Estimated clinical comparability referring to the use in adults and adolescents of the most common inhaled glucocorticosteroids (modified from Ref. [22]).

Inhaled glucocorticosteroid	Total daily dose (mcg)		
	Low	Medium	High
Beclomethasone dipropionate (extrafine)	100–200	>200–400	>400
Budesonide	200–400	>400–800	>800
Ciclesonide	80–160	>160–320	>320
Fluticasone furoate	100	n.a.	200
Fluticasone propionate	100–250	>250–500	>500
Mometasone furoate	110–220	>220–440	>440
Triamcinolone acetanide	400–1000	>1000–2000	>2000

that freely diffuse across the cell membrane, thus binding to a cytoplasmic glucocorticoid receptor (GR) existing as a predominant GR α isoform or as a less common, alternatively spliced and non-functional GR β isoform [23,24]. Budesonide has a high receptor-binding affinity. Indeed, when compared to an arbitrary reference value of 100 assigned to dexamethasone, budesonide displays a relative GR affinity of 935 [25]. Upon binding of GCs to GR, the latter undergoes a conformational change that results in dissociation of anchoring chaperone proteins and exposure of nuclear localization signals, that allow the rapid translocation of the active GR-ligand complex into the nucleus (Fig. 1). Here activated GRs homodimerize and bind to specific genomic DNA sequences called glucocorticoid response elements (GRE) (Fig. 1), located in the regulatory, promoter regions of corticosteroid-inducible genes, which respond to GCs by enhancing their transcription rate [23]. These genes mainly encode anti-inflammatory proteins such as secretory leukoprotease inhibitor (SLPI), mitogen-activated protein kinase phosphatase-1 (MKP-1) and glucocorticoid-induced leucine zipper (GILZ) (Fig. 1), which inhibits the activity of the pro-inflammatory transcription factors nuclear factor- κ B (NF- κ B) and activator protein-1 (AP-1) [24,26]. Acting at GRE level, budesonide exhibits a relevant transcriptional potency, remarkably greater than that shown by other inhaled GCs such as flunisolide and beclomethasone dipropionate [27]. In addition to these mechanisms mediated by GR binding to GRE (receptor protein - DNA interactions), GCs can also implement anti-inflammatory actions induced by protein-protein interactions between activated GRs and NF- κ B or AP-1, resulting in an effective suppression of the pro-inflammatory functions of these transcription factors (Fig. 1). Indeed, NF- κ B and AP-1 are mainly active at sites of airway inflammation in asthma, thus stimulating the expression of multiple genes encoding cytokines, chemokines, enzymes and receptors involved in the pathogenesis of this disease [19]. Budesonide is able to effectively repress the biological activities of both NF- κ B and AP-1 [27].

Besides inhibiting the functions of NF- κ B and AP-1 via transcriptional induction of GILZ and direct protein-protein interactions, GRs can also interfere with the binding of these transcription factors to their consensus nucleotide sequences located within several pro-inflammatory genes. In fact, GRs are able to recruit and activate histone deacetylase-2 (HDAC2) [28], which deacetylates DNA-associated core histones. Histone deacetylation results in a tight winding of genomic DNA around core histones, thereby converting nuclear chromatin in a very compact, closed and repressed molecular structure that impedes the access to their DNA binding sites of NF- κ B and AP-1, as well as of RNA polymerase II and its associated basal factors which are essential to initiate gene transcription. Moreover, HDAC2 also deacetylates GRs, thus enabling them to suppress the activity of pro-inflammatory genes [29]. As a consequence, by recruiting and activating HDAC2, GCs act as powerful repressors of inflammation. In monocyte/macrophage cell lines, as well as in airway epithelial cells, HDAC2 expression is required for the anti-inflammatory effects of budesonide [30].

In addition to the above mentioned mechanisms, GCs are also capable of exerting their effects at a post-transcriptional level. In particular, GCs stimulate the expression of the zinc finger protein tristetraprolin, which destabilizes some cytokine messenger RNAs (mRNAs) by binding to their untranslated 3' ends [31]. This post-transcriptional action of GCs significantly contributes to their inhibitory effects on the synthesis of pro-inflammatory cytokines actively involved in the development and progression of asthma. In human bronchial epithelial cells, budesonide acts as a powerful inducer of tristetraprolin synthesis, and this mechanism results in a significant inhibition of the production of several pro-inflammatory cytokines and chemokines [32].

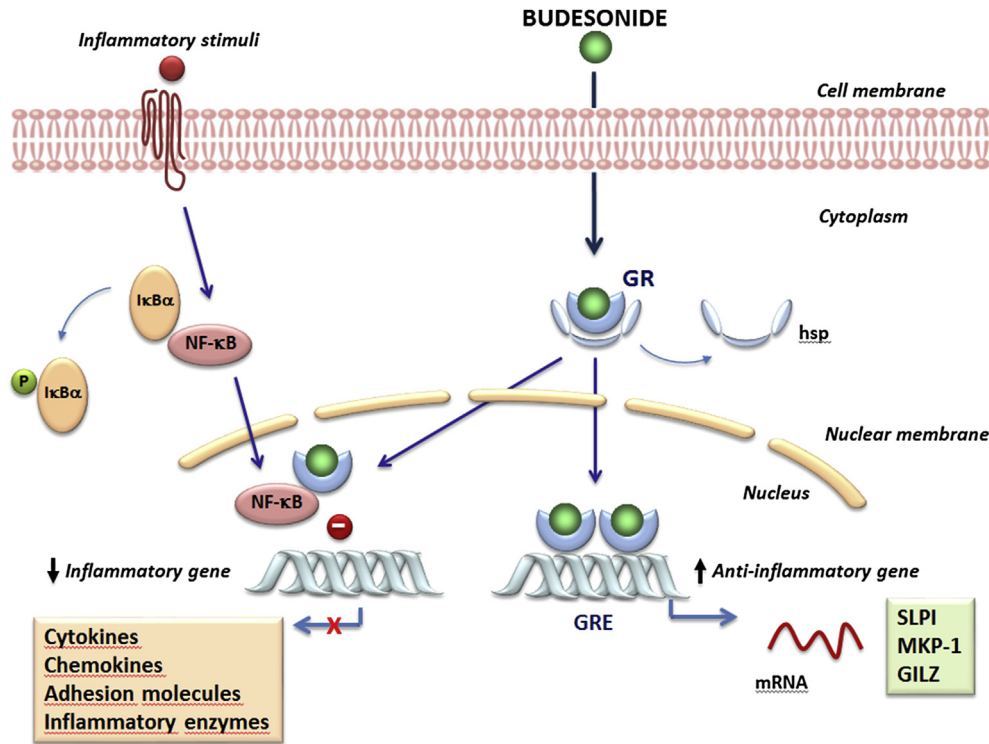


Fig. 1. Molecular mechanisms of budesonide action in asthma. Budesonide is a liposoluble drug that enters the cell and binds to a cytoplasmic glucocorticoid receptor (GR), which in its inactive state is associated with anchoring heat shock proteins (hsp). Upon budesonide binding, GR dissociates from hsp and translocates to the nucleus, where interacts as a dimer with specific DNA sequences named glucocorticoid response elements (GRE). GRE are located within the regulatory regions of target genes, mainly encoding anti-inflammatory proteins such as SLPI (secretory leukoprotease inhibitor), MKP-1 (mitogen-activated protein kinase phosphatase-1) and GILZ (glucocorticoid-induced leucine zipper). Furthermore, activated GR can also bind as a monomer to the transcription factor nuclear factor-κB (NF-κB), activated by inflammatory stimuli that induce its dissociation from the endogenous inhibitor IκB α , which then undergoes phosphorylation and proteolytic degradation. The protein-protein interaction between GR and NF-κB prevents the latter from reaching its consensus nucleotide sequences present in pro-inflammatory genes encoding cytokines, chemokines, adhesion molecules and inflammatory enzymes.

3. Effects of budesonide on airway immune-inflammatory cells

In the airways, budesonide effectively reduces the numbers and inhibits the functions of several immune-inflammatory cells involved in asthma pathobiology, including T lymphocytes, eosinophils, mast cells, and dendritic cells (Fig. 2).

In allergic asthmatics, budesonide enhanced the apoptosis of peripheral blood total lymphocytes, and also decreased antigen-induced proliferation of T cells [33]. Moreover, in these subjects budesonide significantly reduced the percentage of T lymphocytes expressing the co-stimulatory molecule ICOS, which is an important regulator of Th2 activity [33]. These effects translated into a decreased production of Th2 cytokines such as IL-4, IL-5 and IL-13. With regard to CD4⁺ T lymphocytes isolated from induced sputum of patients with allergic asthma, budesonide was capable of significantly suppressing the expression of surface markers of cellular activation, such as class II major histocompatibility complex antigens (HLA-DR) and the α -chain of IL-2 receptor (CD25) [34]. Because of the atopic state of the asthmatic subjects enrolled in this study, it is reasonable to speculate that the main CD4⁺ cellular targets of budesonide were activated Th2 lymphocytes. Furthermore, budesonide is able to markedly improve the activity of Treg cells, whose functions are defective in asthmatic patients. In particular, such effects are mediated by budesonide-induced expression of transcription factor Foxp3 and Toll-like receptors 2 (TLR2) and 4 (TLR4) [33,35]. These effects result in a reduced release of the pro-inflammatory cytokines IL-6 and tumour necrosis factor- α (TNF- α), associated with an increased synthesis of the anti-

inflammatory cytokine IL-10 [35].

Budesonide is a powerful trigger of eosinophil apoptosis, and this pro-apoptotic action represents a key anti-inflammatory effect of inhaled corticosteroids in asthma. In this regard, it is noteworthy that budesonide induced eosinophil apoptosis at clinically relevant drug concentrations [36]. Budesonide elicits the apoptosis of eosinophils by suppressing the synthesis of important survival factors for these cells such as IL-3, IL-5 and granulocyte macrophage-colony stimulating factor (GM-CSF). In addition, using a murine experimental model of allergic asthma, it has been shown that a daily treatment with budesonide significantly attenuated the development of lung eosinophilia in antigen-sensitized animals [37]. This effect originated from a transepithelial eosinophil migration, with subsequent airway clearance. Such a bronchial eosinophil egression was mediated by a remarkable inhibitory action of budesonide on the expression of potent eosinophil chemoattractants, including eotaxin and RANTES (regulated upon activation, normal T cell expressed and secreted) [37]. Budesonide can also decrease eosinophil adhesion to bronchial epithelium. This effect is likely due to budesonide-induced down-regulation of airway epithelial expression of VCAM-1 (vascular cell adhesion molecule-1) [38], an adhesion molecule that interacts with its counter-ligand VLA-4 (very late antigen-4) expressed by eosinophils. In patients with atopic asthma, budesonide drastically reduced eosinophil count in induced sputum, and this effect was associated with a prominent attenuation of airway hyper-responsiveness to methacholine [34]. Furthermore, budesonide was also able to affect the activation state of eosinophils in asthmatic subjects. Indeed, this drug significantly decreased BAL

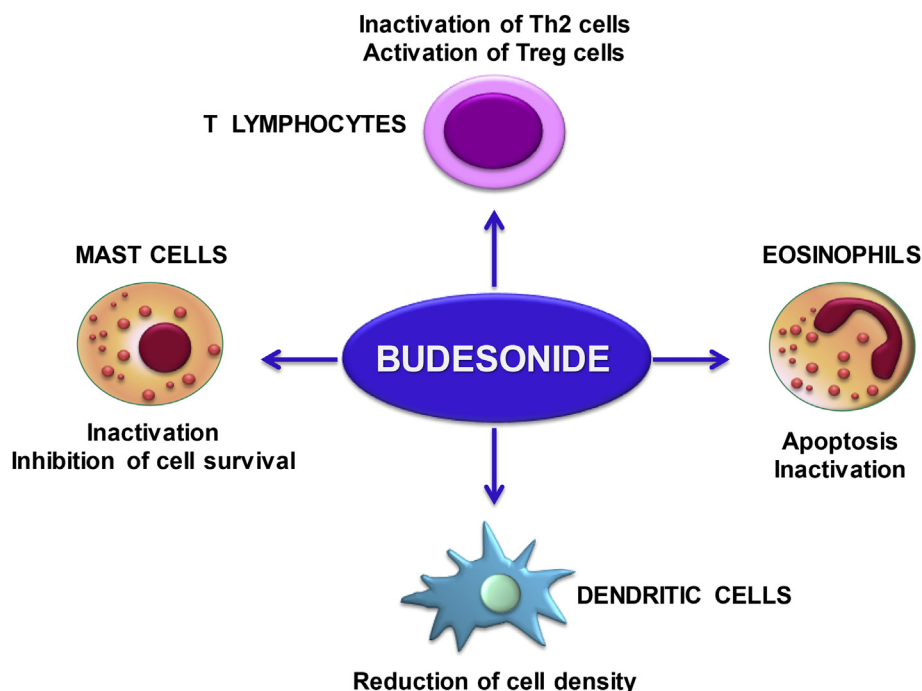


Fig. 2. Effects of budesonide on airway immune-inflammatory cells.

(bronchoalveolar lavage) levels of eosinophilic cationic protein (ECP), one of the main cytotoxic molecules released upon eosinophil degranulation [39].

In human mast cell lines (HMC-1) stimulated by phorbol 12-myristate 13-acetate (PMA) and ionomycin (A23187) to produce IL-4, IL-6, IL-8, GM-CSF and TNF- α , budesonide potently inhibited the release of these cytokines [40]. In particular, the most susceptible cytokine to the inhibitory action of budesonide was IL-4, which plays an essential role in Th2 cell differentiation, as well as in IgE synthesis. Moreover, budesonide is capable of down-regulating IL-1- β -induced expression of stem cell factor (SCF), a very important growth factor for mast cells which acts by promoting the differentiation and chemotaxis of these cells, also inhibiting their apoptosis [41]. Therefore, it is well reasonable that suppression of SCF synthesis by budesonide makes it possible for this drug to significantly reduce both number and activation of mast cells in the airways of asthmatic patients.

In the complex network of immunopathologic circuits underlying asthma a key role is played by dendritic cells, acting as antigen-presenting cells and inducers of Th2 polarization [9]. In this regard, in rat models of bronchial inflammation budesonide has been shown to cause a rapid decrease in both density and activation of airway intraepithelial dendritic cells [42]. These effects can thus significantly contribute to the overall anti-inflammatory activity exerted by budesonide in asthma.

Taken together, the effects of budesonide on the above mentioned cellular targets are mainly responsible for the very effective modulation of inflammation and Th2-driven immune response, provided by this drug in asthmatic airways.

4. Effects of budesonide on airway structural cells

Within the context of bronchial structural changes occurring in asthma, budesonide widely interferes with many pathogenic aspects referring to the involvement of epithelial cells, fibroblasts, airway smooth muscle cells, and vascular endothelial cells (Fig. 3).

In particular, many of the effects elicited by budesonide at level of airway structural cells are due to inhibition of the functions of transforming growth factor- β (TGF- β), a key mediator of airway remodelling that can be produced by bronchial epithelial cells, fibroblasts and eosinophils [43].

In human bronchial epithelial cells, budesonide was capable of fully preventing apoptosis caused by TGF- β [44]. Such an anti-apoptotic action of budesonide was mediated by a complete inhibition of TGF- β -induced, phosphorylation-dependent activation of mitogen-activated protein kinases (MAPK), with special regard to p38 MAPK subgroup [44]. MAPK inhibition by budesonide was likely caused by a GRE-dependent up-regulation of MKP-1 expression [45]. MKP-1 is a phosphatase that dephosphorylates and thus inactivates MAPK. Interestingly, the anti-apoptotic and MAPK-dephosphorylating effects of budesonide were detected in airway epithelial cells using a drug concentration (10^{-8} M) which probably approximates budesonide levels included within the range achieved in the airways after inhalation of therapeutic doses [46]. Therefore, the anti-apoptotic action induced by budesonide on bronchial epithelial cells could significantly contribute to the restorative effect promoted by this drug at level of airway epithelium, which in asthma is characterized by a marked susceptibility to tissue damage and denudation [47]. Indeed, in patients with asthma a 3-month therapy with budesonide enhanced the number of ciliated bronchial epithelial cells [48]. Moreover, budesonide was effective in repairing the damaged airway epithelium of asthmatic subjects undergoing a long-term treatment with this drug [49]. These encouraging findings thereby suggest that it would be valuable to further study the potential effects of inhaled GCs with regard to their regenerative activity exerted on bronchial epithelium in asthma.

Budesonide has been shown to suppress the proliferation of human lung fibroblasts stimulated by TGF- β , and also this effect was mediated by inhibition of MAPK phosphorylation [50]. In these cells, budesonide significantly dampened TGF- β -induced, MAPK-dependent secretion of a potent mitogenic agent for pulmonary

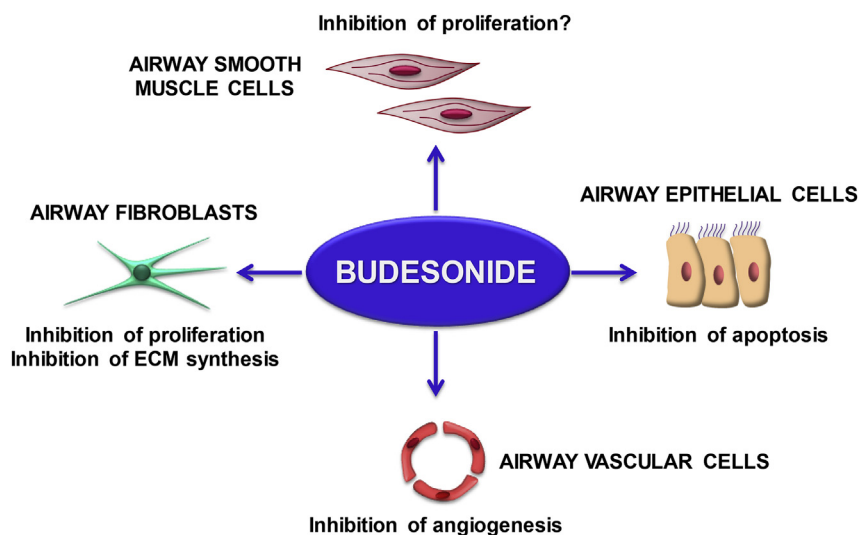


Fig. 3. Effects of budesonide on airway structural cells.

fibroblasts such as IL-11, whose expression levels are quite high in the airways of asthmatic patients [51]. In addition to inhibiting the proliferation of lung fibroblasts, budesonide is also capable of affecting their functions. In particular, bronchial fibroblasts/myofibroblasts are the main cellular sources of the high levels of extracellular matrix (ECM) proteins, including collagen, laminin, tenascin and fibronectin, which cause a thickening of sub-epithelial reticular basement membrane (RBM) in asthmatic airways [52,53]. In this regard, a regular treatment with budesonide decreased tenascin expression in bronchial biopsies taken from patients with allergic asthma [54]. Hence, this effect of budesonide can significantly contribute to its capability of reducing RBM thickness after a long-term inhaled therapy for asthma [55]. Furthermore, budesonide was able to decrease TGF- β -stimulated production of vascular endothelial growth factor (VEGF) by human lung fibroblasts [56]. Because of the central role played by VEGF as a potent pro-angiogenic cytokine [57], the down-regulation of its synthesis promoted by budesonide can reduce the vascular component of airway remodelling in asthma.

Hypertrophy and especially hyperplasia of the bronchial smooth muscle layer are key features of airway remodelling in asthma [58]. In normal airway smooth muscle cells, budesonide inhibited cell proliferation through the interaction of activated GR with the transcription factor C/EBP α (CAAT/enhancer binding protein- α); the subsequent activation of the cell-cycle control gene p21^(waf1/cip1) was responsible for such an anti-proliferative effect [59]. These findings were not confirmed in airway smooth muscle cells from asthmatic subjects, probably because of a defective C/EBP α expression [60,61]. However, the potential anti-proliferative effects of budesonide, eventually involving other cellular mechanisms, need to be further investigated in asthma.

Overall, the effects of budesonide on airway structural cells could provide an effective protection against epithelial damage, associated with a possible reduction of airway wall thickness. Such outcomes might be very useful especially for patients with moderate-to-severe asthma, because in these subjects a regular treatment with budesonide could hopefully attenuate the tendency towards a progressive worsening of airway limitation.

5. Concluding remarks

The excellent therapeutic profile of budesonide as anti-asthma

drug is characterized by a very effective ability to decrease respiratory symptoms and use of rescue bronchodilators, as well as to improve lung function and quality of life, reduce bronchial hyper-responsiveness, and lower the annual rate of disease exacerbations. Such valuable outcomes are closely related to the pleiotropic effects of budesonide, which targets several immune-inflammatory and structural cells in asthmatic airways. Undoubtedly, the core basis of budesonide effectiveness in asthma treatment is strictly linked to its anti-inflammatory and immunosuppressive properties. Indeed, airway cellular infiltration sustained by eosinophils, mast cells, T lymphocytes and dendritic cells, is drastically reduced by budesonide, which also disrupts the intercellular communications mediated by many cytokines, chemokines and other pro-inflammatory mediators. In addition, the powerful anti-inflammatory activity of budesonide is usefully integrated by potential anti-remodelling actions affecting airway structural cells, and these latter aspects probably need to be further studied both *in vitro* and *in vivo*.

Authorship contribution

Girolamo Pelaia, Alessandro Vatrella, Maria Teresa Busceti, Francesco Fabiano, Rosa Terracciano, Maria Gabriella Matera and Rosario Maselli wrote the paper, and drew the figures and the table.

Conflict of interest

- Girolamo Pelaia has received lecture fees and consultancy fees from Almirall, AstraZeneca, Biofutura, Boehringer Ingelheim, Chiesi, Dompè, GlaxoSmithKline, Guidotti-Malesci, Menarini, Mundipharma, Novartis, Teva, Zambon.
- Alessandro Vatrella has received lecture fees and consultancy fees from Almirall, AstraZeneca, Boehringer Ingelheim, Chiesi, Dompè, GlaxoSmithKline, Guidotti-Malesci, Mundipharma, Novartis, Teva.
- Maria Teresa Busceti has no conflict of interest.
- Francesco Fabiano has no conflict of interest.
- Rosa Terracciano has no conflict of interest.
- Maria Gabriella Matera has no conflict of interest.
- Rosario Maselli has received lecture fees and consultancy fees from AstraZeneca, Boehringer Ingelheim, Dompè, GlaxoSmithKline, Guidotti-Malesci, Menarini, Mundipharma, Novartis, Teva.

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