Supplemental Material can be found at:
http://www.jbc.org/content/suppl/2011/09/15/R110.206466.DCAuthor_profile.html

MINIREVIEW

This paper is available online at www.jbc.org

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 286, NO. 38, pp. 32899–32905, September 23, 2011
© 2011 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in the U.S.A.

Biochemical Basis of Asthma Therapy*

Published, JBC Papers in Press, July 28, 2011, DOI 10.1074/jbc.R110.206466

Peter J. Barnes[1]

From the National Heart and Lung Institute, Imperial College, London SW3 6LY, United Kingdom

Current therapy for asthma is highly effective. β_2-Adrenergic receptor (β_2AR) agonists are the most effective bronchodilators and relax airway smooth muscle cells through increased cAMP concentrations and directly opening large conductance Ca^{2+} channels. β_2AR may also activate alternative signaling pathways that may have detrimental effects in asthma. Glucocorticoids are the most effective anti-inflammatory treatments and switch off multiple activated inflammatory genes through recruitment of histone deacetylase-2, activating anti-inflammatory genes, and through increasing mRNA stability of inflammatory genes. There are beneficial molecular interactions between β_2AR and glucocorticoid-activated pathways. Understanding these signaling pathways may lead to even more effective therapies in the future.

Asthma has become one of the most prevalent diseases and is increasing throughout the world, particularly in developing countries. It is characterized by variable airflow obstruction that is secondary to an allergic pattern of inflammation in the airways, which involves infiltration with inflammatory cells (eosinophils and T-helper 2 (T_H2)[2] cells) and the activation of resident mast cells and dendritic cells by allergens (1, 2). Structural cells, such as airway epithelial cells and smooth muscle cells, are important sources of inflammatory mediators as well as activated inflammatory cells. Chronic inflammation may lead to structural changes in the airways, including increased airway smooth muscle cells, fibrosis, angiogenesis, and hyperplasia of mucus-secreting cells. Multiple inflammatory mediators are involved, and many cytokines and chemokines orchestrate this complex chronic inflammation (3).

Although asthma is a complex inflammatory disease, current therapies (if taken correctly) are very effective in the majority of patients (4). There is now a good understanding of how current asthma therapies work at a biochemical level, and this has formed the basis for a search for new treatments (5). Drugs used to treat asthma include bronchodilators, which act mainly by reversing airway smooth muscle contraction, and anti-inflammatory drugs, which suppress inflammation in the airways. The inflammation of asthma is confined to the airways, so inhalational therapy has been found to be the most effective treatment modality and largely avoids systemic side effects.

β_2-Adrenergic Agonists

β_2-Agonists are by far the most effective bronchodilators for asthma, as they act as functional antagonists and relax airway smooth muscle cells whatever the constricting stimulus. Long-acting β_2-agonists (LABA), such as salmeterol and formoterol, have a 12-h duration of action, but once-daily drugs, including indacaterol, vilanterol, and olodaterol, have been developed recently (6). LABA should always be used in combination with a corticosteroid, as they are potentially dangerous if used alone because they do not effectively treat the underlying inflammation.

Bronchodilator Mechanisms—Occupation of β_2-adrenergic receptors (β_2AR) by agonists results in the activation of adenylyl cyclase via the stimulatory G-protein (G_s). This increases intracellular cAMP, leading to activation of PKA, which phosphorylates several target proteins within the cell, leading to activation of myosin light chain phosphatase and inhibition of myosin light chain kinase and thus relaxation of airway smooth muscle (Fig. 1). In addition, β_2-agonists open large conductance calcium-activated potassium channels (BK_{Ca}), which repolarize the smooth muscle cell so that Ca^{2+} is sequestrated into intracellular stores. β_2AR are also *directly* coupled to K_{Ca} via G_s, so relaxation of airway smooth muscle may occur independently of an increase in cAMP. Some actions of β_2-agonists are mediated via other cAMP-regulated proteins, such as EPAC (exchange protein activated by cAMP) (7). For example, the inhibition of airway smooth muscle cell proliferation by β_2-agonists appears to be dependent on EPAC rather than PKA (8).

Airway smooth muscle shows resistance to β_2AR desensitization, and this may be due to a large receptor reserve and a very low level of expression of the enzyme GRK2 (G-protein receptor kinase-2), which phosphorylates and inactivates occupied β_2-receptors (9). IL-1β uncouples pulmonary β_2-receptors in rats *in vivo* by increasing GRK2/5 activity and expression and thus reducing responsiveness to β_2-agonists (10). However, uncertainty remains as to whether β_2AR signaling is abnormal in the airway smooth muscle of asthmatic patients.

Other Airway Effects—β_2-Agonists may have additional effects on airways, as β_2-receptors are localized to several different cells types in the airways. β_2-Agonists may therefore cause bronchodilatation *in vivo* not only via a direct action on airway smooth muscle but also indirectly by inhibiting the release of bronchoconstrictor mediators from inflammatory cells and of neurotransmitters from airway nerves. For example, β_2-agonists inhibit mediator release from mast cells though closing an intermediate conductance Ca^{2+}-activated K^+ channel ($K_{Ca}3.1$) coupled to G_s (11). Stem cell factor, which is secreted by epithelial cells in asthmatic patients, is an important

* This is the fourth article in the Thematic Minireview Series on Molecular Bases of Disease: Asthma. This minireview will be reprinted in the 2011 Minireview Compendium, which will be available in January, 2012.

[1] To whom correspondence should be addressed. E-mail: p.j.barnes@imperial.ac.uk.

[2] The abbreviations used are: T_H2, T-helper 2; LABA, long-acting β_2-agonist(s); β_2AR, β_2-adrenergic receptor(s); PLC, phospholipase C; ACh, acetylcholine; ICS, inhaled corticosteroid(s); GR, glucocorticoid receptor(s); GRE, glucocorticoid response element(s); SLPI, secretory leukoprotease inhibitor; COPD, chronic obstructive pulmonary disease; PDE, phosphodiesterase(s); HDAC, histone deacetylase(s); CysLT, cysteinyl leukotriene(s); 5-LOX, 5′-lipoxygenase; $cPLA_2$, cytosolic phospholipase A_2.

Downloaded from www.jbc.org at Congregazione Figli Immacolata Concezione, on December 13, 2011

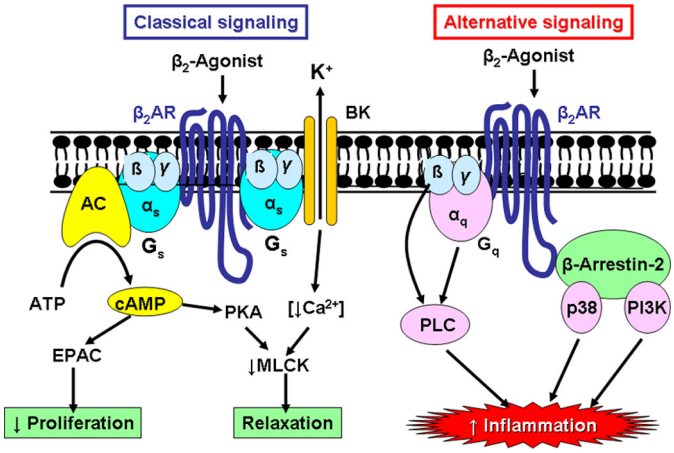

FIGURE 1. **β_2-Agonist signaling pathways.** In the classical pathway, β_2-agonists bind to β_2AR, which are coupled via a stimulatory G-protein (G_s) to adenylyl cyclase (*AC*), resulting in formation of cAMP. cAMP activates PKA, which phosphorylates myosin light chain kinase (*MLCK*) in airway smooth muscle cells, resulting in relaxation. Increased cAMP may also activate EPAC to mediate effects such as inhibition of cell proliferation. β_2AR are also coupled via G_s to a large conductance calcium-activated potassium channels (BK), leading to decreased intercellular Ca^{2+} and inhibition of myosin light chain kinase activation. Alternative signaling by β_2AR may activate $\beta\gamma$-subunits of $G\alpha$ and G_q and via $G\alpha_q$, resulting in activation of PLC. β_2AR also interact with β-arrestin-2, which interacts with p38 MAPK and PI3K. These alternative signaling pathways may increase the expression of inflammatory proteins and therefore may have a deleterious effect in asthma.

factor in keeping mast cells at the airway surface in asthma (2) and counteracts this effect of β_2-agonists (12). Whether β_2-agonists have anti-inflammatory effects in asthma is controversial. The inhibitory effects of β_2-agonists on mast cell mediator release and microvascular leakage are clearly anti-inflammatory, suggesting that β_2-agonists may modify *acute* inflammation. However, β_2-agonists do not have a significant inhibitory effect on the *chronic* inflammation of asthmatic airways, which is suppressed by glucocorticoids. This has now been confirmed by several biopsy and bronchoalveolar lavage studies in patients with asthma who are taking regular β_2-agonists (including LABA), which demonstrate no significant reduction in the number or activation of inflammatory cells in the airways, in contrast to resolution of the inflammation, which occurs with inhaled glucocorticoids (13). This is likely to be related to the fact that β_2-agonist effects on macrophages, eosinophils, and lymphocytes are rapidly desensitized due to a low density of β_2-receptors on these cells and high expression of GRK2 (9). Indeed, exposure to LABA increases the expression of GRK2 and GRK5 in human peripheral lung (14).

β_2-Receptor Polymorphisms—There are several single-nucleotide polymorphisms and haplotypes of the human β_2AR gene (*ADRB2*) that may affect β_2AR function. The common variants are G16R and Q27E, which have *in vitro* effects on receptor desensitization, but clinical studies have shown inconsistent effects on the bronchodilator responses to short- and long-acting β_2-agonists (15). Some studies have shown that patients with the common homozygous Arg^{16}/Arg variant have more frequent adverse effects and a poorer response to short-acting β_2-agonists than heterozygotes or Gly^{16}/Gly homozygotes (16), but, overall, these differences are small, and there appears to be no clinical value in measuring *ADRB2* genotype. No differences have been found with responses to LABA between these genotypes (17).

Alternative Signaling of β_2-Receptors—It is now recognized that, although β_2AR are coupled through G_s to relax airway smooth muscle, they may activate alternative signaling pathways that may have deleterious effects, such as increasing inflammation (Fig. 1). In β_2AR-overexpressing mice, G_q coupled to phospholipase Cβ1 (PLCβ1) is activated, resulting in an enhanced bronchoconstrictor response to mediators, such as ACh and histamine, that signal through G_q (18). $\beta\gamma$-Subunits of G_s may also signal through PLC activation (PLCη2) (19). β-Arrestin-1 and -2 are adaptor proteins involved in uncoupling phosphorylated β_2-receptors from G_s, leading to internalization by clathrin-coated pits. β-Arrestins determine whether β_2-receptors are degraded within the cell by endocytosis or are recycled to the cell membrane (20). Interaction of β_2-receptors with β-arrestin-2 leads to ubiquitination of each protein and subsequent proteasomal destruction (21). As well as terminating receptor function, β-arrestins act as a scaffold to allow receptors to enhance other signaling pathways, such as MAPK and PI3K, independently of G-proteins and therefore allow β_2AR to regulate different responses in the cell (22, 23). This may contribute to the adverse effects of LABA that have been reported (24). Inverse agonists, such as nadolol and carvedilol, block β_2AR and inhibit the signaling of constitutively active β_2AR and paradoxically have been found to have beneficial effects in murine models of asthma. Furthermore, β_2AR knockout mice are protected from development of asthma (25). β_2AR antagonists without inverse agonist activity, such as alprenolol, fail to reverse the asthma phenotype, however. A pilot study in asthma patients showed that, although nadolol reduced lung function in the short-term, there was a reduction in airway hyper-responsiveness after 9 weeks of therapy (26). Deletion of the β-arrestin-2 gene prevents the recruitment of inflammatory cells and airway hyper-responsiveness in mice sensitized and exposed to allergen (27), and both structural and inflammatory cells are involved (28). It is possible that β_2-receptor activation in epithelial cells activates β-arrestin-1/2, leading to activation of proinflammatory kinase pathways, such as p38 MAPK, with the activation of proinflammatory genes (29). Biased β_2-agonists that favor G_s signaling pathways rather than β-arrestin recruitment may prove to be more effective as bronchodilators in the future (20).

Anticholinergics

Anticholinergics are antagonists of muscarinic receptors, and their only action at therapeutic doses is to block the effects of endogenous acetylcholine (ACh). In animals and humans, there is a small degree of resting bronchomotor tone due to tonic vagal nerve impulses that release ACh in the vicinity of airway smooth muscle because it can be blocked by anticholinergic drugs. ACh may also be released from other airway cells, including epithelial and inflammatory cells (30). Airway epithelial cells contain all of the machinery needed for ACh synthesis and release (31, 32). Tiotropium bromide is a once-daily inhaled anticholinergic that dissociates slowly from muscarinic M_1- and M_3-receptors and more rapidly from M_2-receptors. Although β_2-agonists are the most effective

Downloaded from www.jbc.org at Congregazione Figli Immacolata Concezione, on December 13, 2011

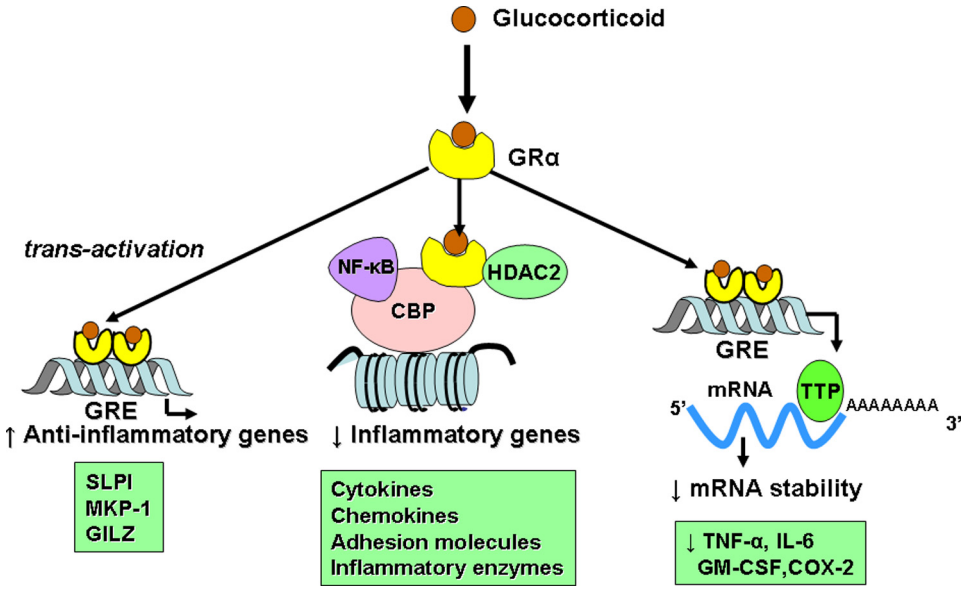

FIGURE 2. **Anti-inflammatory effects of glucocorticoids.** Glucocorticoids cross the cell membrane and bind to GRα in the cytoplasm, which translocates to the nucleus. GR homodimers bind to GRE in glucocorticoid-responsive genes, which may *trans*-activate genes encoding anti-inflammatory proteins, such as SLPI, MKP-1, and glucocorticoid-induced leucine zipper (*GILZ*). GR also interacts with coactivator molecules, such as CBP, which have been activated by proinflammatory transcription factors, such as NF-κB. GR recruits HDAC2, which deacetylates core histones to suppress inflammatory gene transcription. GR also has post-translational effects by increasing the expression of tristetraprolin (*TTP*), which binds to the AU-rich untranslated ends of mRNAs of some inflammatory cytokines, resulting in destabilization and thus reduced expression of these cytokines.

bronchodilators in asthma, anticholinergics may have some additive bronchodilator effect, particularly in patients with severe disease (33). M_3-receptors via G_q and the activation of PLC result in hydrolysis of phosphatidylinositol 4,5-bisphosphate and generation of inositol 1,4,5 trisphosphate, which releases Ca^{2+} from intracellular stores, thus resulting in contraction of airway smooth muscle cells and mucus secretion. M_3-receptors may also be involved in the structural remodeling that occurs in some patients with asthma, as the increase in airway smooth muscle that occurs after chronic allergen exposure in mice is prevented by tiotropium (34). M_2-receptors are also highly expressed in airway smooth muscle cells and inhibit adenylyl cyclase via G_i, thus counteracting the bronchodilator effect of β_2-agonists. M_2-receptors also counteract β_2-agonists by inhibiting K_{Ca} in tracheal smooth muscle cells via $G\beta\gamma$ subunits (35). Presynaptic M_2-receptors that limit ACh release are defective in animal models of asthma, and this may be secondary to eotaxin release from parasympathetic nerves with neural recruitment of eosinophils that release basic proteins to cause M_2-receptor dysfunction (36). There is indirect evidence that presynaptic M_2-receptor function is also impaired in asthmatic patients (37).

Glucocorticoids

Glucocorticoids are by far the most effective therapy for controlling asthma, and inhaled corticosteroids (ICS) have become the mainstay of treatment for all patients with persistent symptoms. There has been major progress in understanding the cellular and molecular mechanisms involved in their anti-inflammatory effects in asthma (38).

Anti-inflammatory Mechanisms—Glucocorticoids diffuse across the cell membrane and bind to glucocorticoid receptors (GR) in the cytoplasm (39, 40). Upon ligand binding, GR are activated and released from chaperone proteins (heat shock protein 90 and others) and rapidly translocate to the nucleus, where they exert their molecular effects. The mechanism of nuclear translocation involves the nuclear import proteins importin-α (karyopherin-β) and importin-13 (41, 42). There is only one form of GR that binds glucocorticoids, termed GRα. GRβ is an alternatively spliced form of GR that interacts with DNA but not with glucocorticoids, so it may theoretically act as a dominant-negative inhibitor of glucocorticoid action by interfering with the binding of GR to DNA (43).

GR homodimerize and bind to glucocorticoid response elements (GRE) in the promoter region of glucocorticoid-responsive genes, and this interaction switches on (or occasionally switches off) gene transcription (Fig. 2). Activation of glucocorticoid-responsive genes occurs via an interaction between the DNA-bound GR and transcriptional coactivator molecules, such as CBP (cAMP-responsive element-binding protein-binding protein), which have intrinsic histone acetyltransferase activity and cause acetylation of core histones (particularly histone 4). This tags histones to recruit chromatin-remodeling engines, such as SWI/SNF, and subsequent association with RNA polymerase II, resulting in gene activation (44, 45). Genes that are switched on by glucocorticoids include genes encoding β_2AR and the anti-inflammatory proteins secretory leukoprotease inhibitor (SLPI) and MKP-1 (MAPK phosphatase-1), which inhibits MAPK pathways. These effects may contribute to the anti-inflammatory actions of glucocorticoids (46, 47). GR interaction with negative GRE or with GRE that cross the transcriptional start site may suppress gene transcription, and this may be important in mediating many of the side effects of glu-

Downloaded from www.jbc.org at Congregazione Figli Immacolata Concezione, on December 13, 2011

cocorticoids, such as inhibition of osteocalcin, which is involved in bone synthesis (48).

The major action of glucocorticoids is to switch off multiple activated inflammatory genes that code for cytokines, chemokines, adhesion molecules, inflammatory enzymes, and receptors (49). These genes are switched on in the airways by proinflammatory transcription factors, such as NF-κB and AP-1 (activator protein-1), both of which are usually activated at sites of inflammation in asthma and chronic obstructive pulmonary disease (COPD), resulting in the switching on of multiple inflammatory genes. These genes are activated through interactions with transcriptional coactivator molecules in a manner similar to that described above for GR-mediated gene transcription (38).

Activated GR interact with corepressor molecules to attenuate NF-κB-associated coactivator activity, thus reducing histone acetylation, chromatin remodeling, and RNA polymerase II actions (38, 44). More importantly, reduction of histone acetylation occurs through the specific recruitment of HDAC2 (histone deacetylase-2) to the activated inflammatory gene complex by activated GR, thereby resulting in effective suppression of activated inflammatory genes within the nucleus (Fig. 2). GR becomes acetylated upon ligand binding, allowing it to bind to GRE, and HDAC2 can target acetylated GR, thereby allowing it to associate with the NF-κB complex (50). The site of acetylation of GR is the lysine-rich region −495 to −492 with the sequence KKTK. Site-directed mutagenesis of Lys^{494} and Lys^{495} prevents GR acetylation and reduces activation of the SLPI gene by glucocorticoids, whereas repression of NF-κB is unaffected.

Additional mechanisms are also important in the anti-inflammatory actions of glucocorticoids. Glucocorticoids have potent inhibitory effects on MAPK signaling pathways through the induction of MKP-1, and this may inhibit the expression of multiple inflammatory genes (Fig. 2) (46, 47). An important effect of glucocorticoids in the treatment of asthma is through suppression of T_H2 cells and T_H2 cytokines (IL-4, IL-5, and IL-13), and this may be mediated via inhibition of the transcription factor GATA3, which regulates the transcription of T_H2 cytokine genes (51). This is controlled by translocation of GATA3 from the cytoplasm to the nucleus via importin-α after phosphorylation by p38 MAPK. Glucocorticoids potently inhibit GATA3 nuclear translocation, as GR competes for nuclear import via importin-α and also induces MKP-1 to reverse the phosphorylation of GATA3 by p38 MAPK (52). A further immunosuppressive effect of glucocorticoids is through enhanced activity and expression of indoleamine 2,3-dioxygenase, a tryptophan-degrading enzyme that plays a key role in the regulation of T lymphocyte function in allergic diseases though increased secretion of the anti-inflammatory cytokine IL-10 (53). Interestingly, this effect of glucocorticoids on indoleamine 2,3-dioxygenase is further enhanced by statins (54).

Post-transcriptional Effects—Some proinflammatory genes, such as TNF-α, have unstable mRNA that is rapidly degraded by certain RNases but stabilized when cells are stimulated by inflammatory mediators. Glucocorticoids reverse this effect, resulting in rapid degradation of mRNA and reduced inflammatory protein secretion (55). This may be mediated through the increased gene expression of proteins that destabilize mRNAs of inflammatory proteins, such as the zinc finger protein tristetraprolin, which binds to the AU-rich 3′-untranslated region of mRNAs of certain cytokine mRNAs (Fig. 2) (56).

Interaction with β_2-Adrenergic Receptors—Inhaled β_2-agonists and glucocorticoids are frequently used together (usually as a fixed-combination inhaler of a glucocorticoid with a LABA), and it is now recognized that there are important biochemical interactions between these two classes of drug (57, 58). Glucocorticoids increase transcription of the β_2-receptor gene, resulting in increased expression of cell surface receptors. This has been demonstrated in human lung *in vitro* (59) and nasal mucosa *in vivo* (60) after topical application of a glucocorticoid. In this way, glucocorticoids protect against the down-regulation of β_2-receptors after long-term administration (61). This may be important for the non-bronchodilator effects of β_2-agonists, such as mast cell stabilization. Glucocorticoids may also enhance the coupling of β_2-receptors to G_s, thus enhancing β_2-agonist effects and reversing the uncoupling of β_2-receptors that may occur in response to inflammatory mediators, such as IL-1β, through a stimulatory effect on GRK2 (14).

There is increasing evidence that β_2-agonists may affect GR function and thus enhance the anti-inflammatory effects of glucocorticoids. LABA increase the translocation of GR from the cytoplasm to the nucleus after activation by glucocorticoids (62). This effect has been demonstrated in sputum macrophages of asthmatic patients after treatment with an inhaled glucocorticoid and inhaled LABA (63). This suggests that LABA and glucocorticoids enhance each other's beneficial effects in asthma therapy, and this may contribute to the greater efficacy of combination inhalers compared with increased doses of ICS in clinical trials (64).

Glucocorticoid Resistance Pathways—Patients with severe asthma and asthmatics who smoke have a poor response to glucocorticoids, which necessitates the need for high doses, and a few patients are completely resistant (65). Several biochemical mechanisms have now been identified to account for glucocorticoid resistance. In smoking asthmatics and patients with severe asthma, there is a reduction in HDAC2 activity and expression, which prevents glucocorticoids from switching off activated inflammatory genes (66, 67). This reduction in HDAC2 may be secondary to oxidative and nitrative stress and the generation of peroxynitrite, which nitrates critical tyrosine residues on HDAC2, leading to its ubiquitination and proteasomal degradation (Fig. 3) (68). Oxidative stress activates PI3Kδ, which results in subsequent HDAC2 phosphorylation and inactivation (69). Hypoxia reduces transcription of the *HDAC2* gene via activation of hypoxia-inducible factor-1α, which binds to the promoter region, leading to transcriptional repression (70). Other mechanisms may also contribute to glucocorticoid insensitivity, including reduced translocation of GR as a result of phosphorylation by p38 MAPK (71) and JNK, which phosphorylates GR at Ser^{226} (72). Some asthmatic patients with severe glucocorticoid resistance show abnormal histone acetylation patterns (73). Another proposed mechanism is that increased GRβ may prevent GRα binding to

Downloaded from www.jbc.org at Congregazione Figli Immacolata Concezione, on December 13, 2011

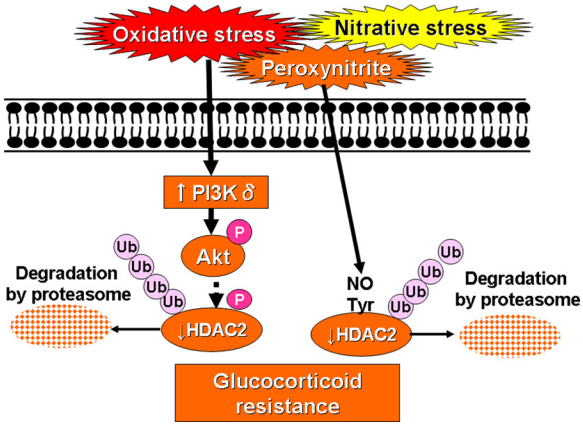

FIGURE 3. **Signaling pathways involved in glucocorticoid resistance in severe asthma and smoking asthmatics.** Oxidative and nitrative stress generates peroxynitrite, which nitrates specific tyrosine residues (*NO-Tyr*) on HDAC2, resulting in its ubiquitination (*Ub*) and proteasomal degradation. Oxidative stress also activates PI3Kδ, which also leads to subsequent phosphorylation (*P*) and ubiquitination of HDAC2, resulting in glucocorticoid resistance.

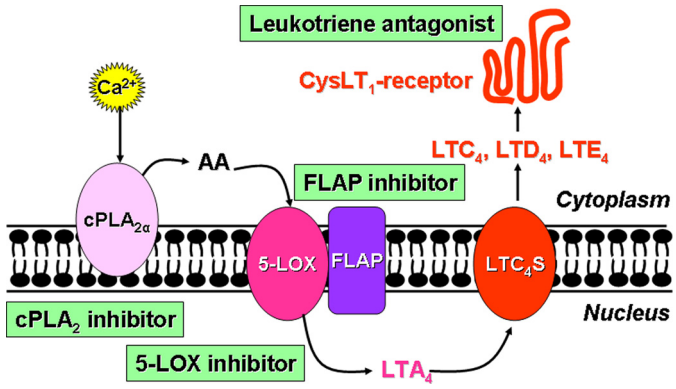

FIGURE 4. **Leukotriene modifiers.** Cell activation increases intracellular Ca^{2+} ions, leading to association of $cPLA_2\alpha$ with the nuclear membrane, generating arachidonic acid. This activates 5-LOX, which is also located in the nuclear membrane bound to FLAP. This generates LTA_4, which is converted by membrane-bound LTC_4 synthase (*LTC_4S*) in cells, such as mast cells and eosinophils, into LTC_4, LTD_4, and LTE_4, which then activate $CysLT_1$ receptors on target cells, such as airway smooth muscle cells. This pathway may be blocked by various types of drugs shown in *green boxes*.

DNA, but the amounts of GRβ appear to be too low (74). Although glucocorticoids do not bind to GRβ, it is transcriptionally active, and the GR antagonist mifepristone (RU-486) binds to GRβ, making it translocate to the nucleus, but the endogenous ligand of GRβ is currently unidentified (75).

Theophylline

Methylxanthines, such as theophylline, which are related to caffeine, have been used in the treatment of asthma since 1930, and theophylline is still widely used in developing countries because it is inexpensive. However, the frequency of side effects and the relatively low efficacy of theophylline have recently led to reduced usage in many countries because inhaled β_2-agonists are more effective as bronchodilators and ICS have greater anti-inflammatory effects. In patients with severe asthma, theophylline still remains a very useful add-on therapy, and there is increasing evidence that it has anti-inflammatory effects and may enhance the anti-inflammatory effects of glucocorticoids (76).

The mechanism of action of theophylline is still uncertain. The bronchodilator effect seen at high plasma concentrations (10–20 mg/liter) is due to inhibition of phosphodiesterases (PDE) in airway smooth muscle, particularly PDE3, which results in increased cAMP concentrations. Inhibition of PDE4 accounts for the common side effects of nausea, diarrhea, and headaches. At therapeutic concentrations, theophylline antagonizes adenosine receptors, particularly A_{2B}-receptors, on mast cells to inhibit adenosine-mediated mediator release (77). Antagonism of A_1-receptors may account for the serious side effects of cardiac arrhythmias and seizures. Theophylline prevents the translocation of NF-κB into the nucleus, thus potentially reducing the expression of inflammatory genes in asthma, and this appears to be due to a protective effect against the degradation of the inhibitory protein IκBα (78). However, these effects are seen only at high concentrations and may be mediated by inhibition of PDE. Theophylline promotes apoptosis in eosinophils and neutrophils *in vitro*. This is associated with a reduction in the anti-apoptotic protein Bcl-2 (79). This effect is not mediated via PDE inhibition but, in neutrophils, may be mediated by antagonism of adenosine A_{2A}-receptors (80).

Theophylline is an activator of histone deacetylases (HDAC) at low therapeutic concentrations (1–5 mg/liter), thus enhancing the anti-inflammatory effects of glucocorticoids *in vitro* and in animal models *in vivo* (81, 82). This mechanism is independent of PDE inhibition or adenosine antagonism and appears to be mediated *in vitro* and *in vivo* by direct inhibition of oxidant-activated PI3Kδ, which is activated by oxidative stress (69, 83). The anti-inflammatory effects of theophylline are inhibited by the HDAC inhibitor trichostatin A. Low doses of theophylline increase HDAC activity in bronchial biopsies of asthmatic patients and correlate with reduction in eosinophils in the airway wall (81).

Leukotriene Modifiers

Cysteinyl leukotrienes (CysLT) are produced in asthma and have potent effects on airway function, inducing bronchoconstriction, plasma exudation, and mucus secretion, and possibly on eosinophilic inflammation (84). Blocking leukotriene pathways may be beneficial in asthma, and this has led to the development of 5′-lipoxygenase (5-LOX) enzyme inhibitors (of which zileuton is the only drug marketed) and several antagonists of the $CysLT_1$ receptor, including montelukast, zafirlukast, and pranlukast. Although there are clinical improvements in symptoms and lung function and reduced exacerbations, these drugs are significantly less effective than low doses of ICS in asthma but are used because they are effective orally and appear to be safe. The pathways involved in CysLT synthesis are strictly compartmentalized within the cell. Ca^{2+} stimulates the binding of type IVA cytosolic phospholipase A_2 ($cPLA_2\alpha$) to the cell membrane, with release of arachidonic acid, and the binding of 5-LOX to the nuclear membrane, where it co-localizes with FLAP (five-LOX-activating protein) and converts arachidonic acid to LTA_4 (Fig. 4). In certain cells that express LTC_4 synthase (mast cells and eosinophils), 5-LOX also co-localizes with these enzymes to generate CysLT from LTA_4 (85). FLAP, LTC_4 synthase, and PLA_2 inhibitors are now in development as

Downloaded from www.jbc.org at Congregazione Figli Immacolata Concezione, on December 13, 2011

potential asthma therapies. Secreted PLA_2 enzymes cooperate with $cPLA_2$; secreted PLA_2-X is increased in the airways of asthmatic patients and potently stimulates CysLT release from eosinophils, which is inhibited by p38 MAPK and JNK inhibitors (86).

Concluding Remarks

There is now a relatively good understanding of how drugs used to treat asthma work in terms of their biochemical mechanisms, providing opportunities to improve existing treatments and to discover novel therapies in the future (5). For example, once-daily β_2-agonists, such as indacaterol and vilanterol, that dissociate more slowly from β_2AR have been developed (6). The recognition that β_2-agonists may have potentially adverse effects through activating inflammatory pathways via interaction with β-arrestins might lead to the development of biased β_2-agonists that are less likely to have this activity and, at the same time, are less likely to result in tolerance (20). Several once-daily anticholinergics have been developed for treating COPD, but they may also be useful in treating patients with severe asthma (33). There are additive effects between β_2-agonists and anticholinergics, which may be explained by cross-talk between signaling pathways, such that inhibition of M_2-receptors may enhance the stimulatory effect of β_2-agonists on adenylyl cyclase, and inhibition of phosphatidylinositol hydrolysis via M_3-receptors may also increase signaling effects of β_2-agonists. This has led to the development of fixed-combination inhalers that contain a once-daily β_2-agonist and anticholinergic (87).

Understanding the biochemical pathways involved in suppression of inflammation by glucocorticoids and the molecular mechanisms of glucocorticoid resistance has led to new therapeutic approaches. Nonsteroidal selective glucocorticoid receptor agonists (so-called dissociated steroids) that target the *trans*-repression pathway linked to inhibition of NF-κB-induced inflammatory genes with relative sparing of *trans*-activation pathways that involve DNA binding should theoretically reduce the side effects of glucocorticoids that appear to be mediated mainly via *trans*-activation (88). In reality, it has been difficult to demonstrate marked dissociation of beneficial and adverse effects with the compounds currently in development. Approaches to treating glucocorticoid resistance in asthma include alternative anti-inflammatory treatments such as MAPK inhibitors and drugs that activate HDAC2, such as theophylline and other PI3Kδ inhibitors. PDE4 inhibitors (now approved in Europe for severe COPD) may be useful in severe asthma (89). Although leukotriene receptor antagonists have been disappointing in the treatment of asthma, drugs acting higher up the leukotriene synthesis pathway, such as 5-LOX and subtype-selective PLA_2 inhibitors, might be more effective through inhibiting the synthesis of more inflammatory mediators (90). It has so far proved difficult to development novel anti-inflammatory treatments for asthma that are as safe or effective as ICS, but perhaps a better understanding of the inflammatory signaling pathways in asthmatic airway cells might lead to more effective novel classes of therapy in the future.

REFERENCES

1. Barnes, P. J. (2008) *Nat. Immunol. Rev.* **8,** 183–192
2. Hamid, Q., and Tulic, M. (2009) *Annu. Rev. Physiol.* **71,** 489–507
3. Barnes, P. J. (2008) *J. Clin. Invest.* **118,** 3546–3556
4. Bateman, E. D., Hurd, S. S., Barnes, P. J., Bousquet, J., Drazen, J. M., FitzGerald, M., Gibson, P., Ohta, K., O'Byrne, P., Pedersen, S. E., Pizzichini, E., Sullivan, S. D., Wenzel, S. E., and Zar, H. J. (2008) *Eur. Respir. J.* **31,** 143–178
5. Barnes, P. J. (2010) *Trends Pharmacol. Sci.* **31,** 335–343
6. Cazzola, M., and Matera, M. G. (2009) *Eur. Respir. J.* **34,** 757–769
7. Holz, G. G., Kang, G., Harbeck, M., Roe, M. W., and Chepurny, O. G. (2006) *J. Physiol.* **577,** 5–15
8. Kassel, K. M., Wyatt, T. A., Panettieri, R. A., Jr., and Toews, M. L. (2008) *Am. J. Physiol. Lung Cell. Mol. Physiol.* **294,** L131–L138
9. McGraw, D. W., and Liggett, S. B. (1997) *J. Biol. Chem.* **272,** 7338–7344
10. Mak, J. C., Hisada, T., Salmon, M., Barnes, P. J., and Chung, K. F. (2002) *Br. J. Pharmacol.* **135,** 987–996
11. Duffy, S. M., Cruse, G., Lawley, W. J., and Bradding, P. (2005) *FASEB J.* **19,** 1006–1008
12. Cruse, G., Yang, W., Duffy, S. M., Chachi, L., Leyland, M., Amrani, Y., and Bradding, P. (2010) *J. Allergy Clin. Immunol.* **125,** 257–263
13. Howarth, P. H., Beckett, P., and Dahl, R. (2000) *Respir. Med.* **94,** S22–S25
14. Mak, J. C., Chuang, T. T., Harris, C. A., and Barnes, P. J. (2002) *Eur. J. Pharmacol.* **436,** 165–172
15. Hawkins, G. A., Weiss, S. T., and Bleecker, E. R. (2008) *Pharmacogenomics* **9,** 349–358
16. Israel, E., Chinchilli, V. M., Ford, J. G., Boushey, H. A., Cherniack, R., Craig, T. J., Deykin, A., Fagan, J. K., Fahy, J. V., Fish, J., Kraft, M., Kunselman, S. J., Lazarus, S. C., Lemanske, R. F., Jr., Liggett, S. B., Martin, R. J., Mitra, N., Peters, S. P., Silverman, E., Sorkness, C. A., Szefler, S. J., Wechsler, M. E., Weiss, S. T., and Drazen, J. M. (2004) *Lancet* **364,** 1505–1512
17. Bleecker, E. R., Postma, D. S., Lawrance, R. M., Meyers, D. A., Ambrose, H. J., and Goldman, M. (2007) *Lancet* **370,** 2118–2125
18. McGraw, D. W., Almoosa, K. F., Paul, R. J., Kobilka, B. K., and Liggett, S. B. (2003) *J. Clin. Invest.* **112,** 619–626
19. Zhou, Y., Sondek, J., and Harden, T. K. (2008) *Biochemistry* **47,** 4410–4417
20. Violin, J. D., and Lefkowitz, R. J. (2007) *Trends Pharmacol. Sci.* **28,** 416–422
21. Shenoy, S. K., McDonald, P. H., Kohout, T. A., and Lefkowitz, R. J. (2001) *Science* **294,** 1307–1313
22. Schmid, C. L., and Bohn, L. M. (2009) *Pharmacol. Ther.* **121,** 285–293
23. DeWire, S. M., Ahn, S., Lefkowitz, R. J., and Shenoy, S. K. (2007) *Annu. Rev. Physiol.* **69,** 483–510
24. Nelson, H. S., and Dorinsky, P. M. (2006) *Ann. Intern. Med.* **145,** 706–710
25. Nguyen, L. P., Lin, R., Parra, S., Omoluabi, O., Hanania, N. A., Tuvim, M. J., Knoll, B. J., Dickey, B. F., and Bond, R. A. (2009) *Proc. Natl. Acad. Sci. U.S.A.* **106,** 2435–2440
26. Hanania, N. A., Singh, S., El-Wali, R., Flashner, M., Franklin, A. E., Garner, W. J., Dickey, B. F., Parra, S., Ruoss, S., Shardonofsky, F., O'Connor, B. J., Page, C., and Bond, R. A. (2008) *Pulm. Pharmacol. Ther.* **21,** 134–141
27. Walker, J. K., Fong, A. M., Lawson, B. L., Savov, J. D., Patel, D. D., Schwartz, D. A., and Lefkowitz, R. J. (2003) *J. Clin. Invest.* **112,** 566–574
28. Hollingsworth, J. W., Theriot, B. S., Li, Z., Lawson, B. L., Sunday, M., Schwartz, D. A., and Walker, J. K. (2010) *Am. J. Respir. Cell Mol. Biol.* **43,** 269–275
29. Gong, K., Li, Z., Xu, M., Du, J., Lv, Z., and Zhang, Y. (2008) *J. Biol. Chem.* **283,** 29028–29036
30. Wessler, I., and Kirkpatrick, C. J. (2008) *Br. J. Pharmacol.* **154,** 1558–1571
31. Kummer, W., Lips, K. S., and Pfeil, U. (2008) *Histochem. Cell Biol.* **130,** 219–234
32. Bateman, E. D., Rennard, S., Barnes, P. J., Dicpinigaitis, P. V., Gosens, R., Gross, N. J., Nadel, J. A., Pfeifer, M., Racké, K., Rabe, K. F., Rubin, B. K., Welte, T., and Wessler, I. (2009) *Pulm. Pharmacol. Ther.* **22,** 533–542
33. Peters, S. P., Kunselman, S. J., Icitovic, N., Moore, W. C., Pascual, R., Ameredes, B. T., Boushey, H. A., Calhoun, W. J., Castro, M., Cherniack, R. M., Craig, T., Denlinger, L., Engle, L. L., DiMango, E. A., Fahy, J. V.,

Downloaded from www.jbc.org at Congregazione Figli Immacolata Concezione, on December 13, 2011

Israel, E., Jarjour, N., Kazani, S. D., Kraft, M., Lazarus, S. C., Lemanske, R. F., Jr., Lugogo, N., Martin, R. J., Meyers, D. A., Ramsdell, J., Sorkness, C. A., Sutherland, E. R., Szefler, S. J., Wasserman, S. I., Walter, M. J., Wechsler, M. E., Chinchilli, V. M., and Bleecker, E. R. (2010) *N. Engl. J. Med.* **363,** 1715–1726
34. Bos, I. S., Gosens, R., Zuidhof, A. B., Schaafsma, D., Halayko, A. J., Meurs, H., and Zaagsma, J. (2007) *Eur. Respir. J.* **30,** 653–661
35. Zhou, X. B., Wulfsen, I., Lutz, S., Utku, E., Sausbier, U., Ruth, P., Wieland, T., and Korth, M. (2008) *J. Biol. Chem.* **283,** 21036–21044
36. Fryer, A. D., Stein, L. H., Nie, Z., Curtis, D. E., Evans, C. M., Hodgson, S. T., Jose, P. J., Belmonte, K. E., Fitch, E., and Jacoby, D. B. (2006) *J. Clin. Invest.* **116,** 228–236
37. Minette, P. A., Lammers, J. W., Dixon, C. M., McCusker, M. T., and Barnes, P. J. (1989) *J. Appl. Physiol.* **67,** 2461–2465
38. Barnes, P. J. (2006) *Br. J. Pharmacol.* **148,** 245–254
39. Rhen, T., and Cidlowski, J. A. (2005) *N. Engl. J. Med.* **353,** 1711–1723
40. Nicolaides, N. C., Galata, Z., Kino, T., Chrousos, G. P., and Charmandari, E. (2010) *Steroids* **75,** 1–12
41. Goldfarb, D. S., Corbett, A. H., Mason, D. A., Harreman, M. T., and Adam, S. A. (2004) *Trends Cell Biol.* **14,** 505–514
42. Tao, T., Lan, J., Lukacs, G. L., Haché, R. J., and Kaplan, F. (2006) *Am. J. Respir. Cell Mol. Biol.* **35,** 668–680
43. Lewis-Tuffin, L. J., and Cidlowski, J. A. (2006) *Ann. N.Y. Acad. Sci.* **1069,** 1–9
44. Ito, K., Barnes, P. J., and Adcock, I. M. (2000) *Mol. Cell. Biol.* **20,** 6891–6903
45. John, S., Sabo, P. J., Johnson, T. A., Sung, M. H., Biddie, S. C., Lightman, S. L., Voss, T. C., Davis, S. R., Meltzer, P. S., Stamatoyannopoulos, J. A., and Hager, G. L. (2008) *Mol. Cell* **29,** 611–624
46. Barnes, P. J. (2006) *Eur. Respir. J.* **27,** 413–426
47. Clark, A. R. (2003) *J. Endocrinol.* **178,** 5–12
48. Dostert, A., and Heinzel, T. (2004) *Curr. Pharm. Des.* **10,** 2807–2816
49. Barnes, P. J., and Adcock, I. M. (2003) *Ann. Intern. Med.* **139,** 359–370
50. Ito, K., Yamamura, S., Essilfie-Quaye, S., Cosio, B., Ito, M., Barnes, P. J., and Adcock, I. M. (2006) *J. Exp. Med.* **203,** 7–13
51. Maneechotesuwan, K., Xin, Y., Ito, K., Jazrawi, E., Lee, K. Y., Usmani, O. S., Barnes, P. J., and Adcock, I. M. (2007) *J. Immunol.* **178,** 2491–2498
52. Maneechotesuwan, K., Yao, X., Ito, K., Jazrawi, E., Usmani, O. S., Adcock, I. M., and Barnes, P. J. (2009) *PLoS Med.* **6,** e1000076
53. Maneechotesuwan, K., Supawita, S., Kasetsinsombat, K., Wongkajornsilp, A., and Barnes, P. J. (2008) *J. Allergy Clin. Immunol.* **121,** 43–50
54. Maneechotesuwan, K., Ekjiratrakul, W., Kasetsinsombat, K., Wongkajornsilp, A., and Barnes, P. J. (2010) *J. Allergy Clin. Immunol.* **126,** 754–762
55. Bergmann, M. W., Staples, K. J., Smith, S. J., Barnes, P. J., and Newton, R. (2004) *Am. J. Respir. Cell Mol. Biol.* **30,** 555–563
56. Smoak, K., and Cidlowski, J. A. (2006) *Mol. Cell. Biol.* **26,** 9126–9135
57. Barnes, P. J. (2002) *Eur. Respir. J.* **19,** 182–191
58. Newton, R., Leigh, R., and Giembycz, M. A. (2010) *Pharmacol. Ther.* **125,** 286–327
59. Mak, J. C., Nishikawa, M., and Barnes, P. J. (1995) *Am. J. Physiol.* **268,** L41–L46
60. Baraniuk, J. N., Ali, M., Brody, D., Maniscalco, J., Gaumond, E., Fitzgerald, T., Wong, G., Yuta, A., Mak, J. C., Barnes, P. J., Bascom, R., and Troost, T. (1997) *Am. J. Respir. Crit. Care Med.* **155,** 704–710
61. Mak, J. C., Nishikawa, M., Shirasaki, H., Miyayasu, K., and Barnes, P. J. (1995) *J. Clin. Invest.* **96,** 99–106
62. Roth, M., Johnson, P. R., Rüdiger, J. J., King, G. G., Ge, Q., Burgess, J. K., Anderson, G., Tamm, M., and Black, J. L. (2002) *Lancet* **360,** 1293–1299
63. Usmani, O. S., Ito, K., Maneechotesuwan, K., Ito, M., Johnson, M., Barnes, P. J., and Adcock, I. M. (2005) *Am. J. Respir. Crit. Care Med.* **172,** 704–712
64. Gibson, P. G., Powell, H., and Ducharme, F. M. (2007) *J. Allergy Clin. Immunol.* **119,** 344–350
65. Barnes, P. J., and Adcock, I. M. (2009) *Lancet* **373,** 1905–1917
66. Hew, M., Bhavsar, P., Torrego, A., Meah, S., Khorasani, N., Barnes, P. J., Adcock, I., and Chung, K. F. (2006) *Am. J. Respir. Crit. Care Med.* **174,** 134–141
67. Barnes, P. J. (2009) *Annu. Rev. Physiol.* **71,** 451–464
68. Osoata, G. O., Yamamura, S., Ito, M., Vuppusetty, C., Adcock, I. M., Barnes, P. J., and Ito, K. (2009) *Biochem. Biophy. Res. Commun.* **384,** 366–371
69. To, Y., Ito, K., Kizawa, Y., Failla, M., Ito, M., Kusama, T., Elliott, W. M., Hogg, J. C., Adcock, I. M., and Barnes, P. J. (2010) *Am. J. Resp. Crit. Care Med.* **182,** 897–904
70. Charron, C. E., Chou, P. C., Coutts, D. J., Kumar, V., To, M., Akashi, K., Pinhu, L., Griffiths, M., Adcock, I. M., Barnes, P. J., and Ito, K. (2009) *J. Biol. Chem.* **284,** 36047–36054
71. Irusen, E., Matthews, J. G., Takahashi, A., Barnes, P. J., Chung, K. F., and Adcock, I. M. (2002) *J. Allergy Clin. Immunol.* **109,** 649–657
72. Ismaili, N., and Garabedian, M. J. (2004) *Ann. N.Y. Acad. Sci.* **1024,** 86–101
73. Matthews, J. G., Ito, K., Barnes, P. J., and Adcock, I. M. (2004) *J. Allergy Clin. Immunol.* **113,** 1100–1108
74. Pujols, L., Mullol, J., and Picado, C. (2007) *Curr. Allergy Asthma Rep.* **7,** 93–99
75. Lewis-Tuffin, L. J., Jewell, C. M., Bienstock, R. J., Collins, J. B., and Cidlowski, J. A. (2007) *Mol. Cell. Biol.* **27,** 2266–2282
76. Barnes, P. J. (2003) *Am. J. Respir. Crit. Care Med.* **167,** 813–818
77. Wilson, C. N. (2008) *Br. J. Pharmacol.* **155,** 475–486
78. Ichiyama, T., Hasegawa, S., Matsubara, T., Hayashi, T., and Furukawa, S. (2001) *Naunyn-Schmiedebergs Arch. Pharmacol.* **364,** 558–561
79. Chung, I. Y., Nam-Kung, E. K., Lee, N. M., Chang, H. S., Kim, D. J., Kim, Y. H., and Park, C. S. (2000) *Cell. Immunol.* **203,** 95–102
80. Yasui, K., Agematsu, K., Shinozaki, K., Hokibara, S., Nagumo, H., Nakazawa, T., and Komiyama, A. (2000) *J. Leukocyte Biol.* **67,** 529–535
81. Ito, K., Lim, S., Caramori, G., Cosio, B., Chung, K. F., Adcock, I. M., and Barnes, P. J. (2002) *Proc. Natl. Acad. Sci. U.S.A.* **99,** 8921–8926
82. Cosio, B. G., Tsaprouni, L., Ito, K., Jazrawi, E., Adcock, I. M., and Barnes, P. J. (2004) *J. Exp. Med.* **200,** 689–695
83. Marwick, J. A., Caramori, G., Stevenson, C. S., Casolari, P., Jazrawi, E., Barnes, P. J., Ito, K., Adcock, I. M., Kirkham, P. A., and Papi, A. (2009) *Am. J. Respir. Crit. Care Med.* **179,** 542–548
84. Peters-Golden, M., and Henderson, W. R., Jr. (2007) *N. Engl. J. Med.* **357,** 1841–1854
85. Newcomer, M. E., and Gilbert, N. C. (2010) *J. Biol. Chem.* **285,** 25109–25114
86. Lai, Y., Oslund, R. C., Bollinger, J. G., Henderson, W. R., Jr., Santana, L. F., Altemeier, W. A., Gelb, M. H., and Hallstrand, T. S. (2010) *J. Biol. Chem.* **285,** 41491–41500
87. van Noord, J. A., Buhl, R., Laforce, C., Martin, C., Jones, F., Dolker, M., and Overend, T. (2010) *Thorax* **65,** 1086–1091
88. Schäcke, H., Berger, M., Rehwinkel, H., and Asadullah, K. (2007) *Mol. Cell. Endocrinol.* **275,** 109–117
89. Hatzelmann, A., Morcillo, E. J., Lungarella, G., Adnot, S., Sanjar, S., Beume, R., Schudt, C., and Tenor, H. (2010) *Pulm. Pharmacol. Ther.* **23,** 235–256
90. Garcia-Garcia, H. M., and Serruys, P. W. (2009) *Curr. Opin. Lipidol.* **20,** 327–332

Downloaded from www.jbc.org at Congregazione Figli Immacolata Concezione, on December 13, 2011