Molecular mechanisms in allergy and clinical immunology
(Supported by an unrestricted educational grant from Genentech, Inc. and Novartis Pharmaceuticals Corporation)

Series editors: Joshua A. Boyce, MD, Fred Finkelman, MD, William T. Shearer, MD, PhD, and Donata Vercelli, MD

Glucocorticoid actions on airway epithelial responses in immunity: Functional outcomes and molecular targets

Cristiana Stellato, MD, PhD *Baltimore, Md*

This activity is available for CME credit. See page 32A for important information.

Research on the biology of airway epithelium in the last decades has progressively uncovered the many roles of this cell type during the immune response. Far from the early view of the epithelial layer simply as a passive barrier, the airway epithelium is now considered a central player in mucosal immunity, providing innate mechanisms of first-line host defense as well as facilitating adaptive immune responses. Alterations of the epithelial phenotype are primarily involved in the pathogenesis of allergic airways disease, particularly in severe asthma. Appreciation of the epithelium as target of glucocorticoid therapy has also grown, because of studies defining the pathways and mediators affected by glucocorticoids, and studies illustrating the relevance of the control of the response from epithelium in the overall efficacy of topical and systemic therapy with glucocorticoids. Studies of the mechanism of action of glucocorticoids within the biology of the immune response of the epithelium have uncovered mechanisms of gene regulation involving both transcriptional and posttranscriptional events. The view of epithelium as therapeutic target therefore has plenty of room to evolve, as new knowledge on the role of epithelium in immunity is established and novel pathways mediating glucocorticoid regulation are elucidated. (J Allergy Clin Immunol 2007;120:1247-63.)

Key words: *Airway epithelium, asthma, glucocorticoids, immunity, inflammation, inhaled corticosteroid therapy*

Abbreviations used

AP-1:	Activator protein 1
ARE:	Adenylate-urydilate-rich element
BRF:	Butyrate response factor
DC:	Dendritic cell
EGFR:	Epithelial growth factor receptor
Erk:	Extracellular signal-regulated kinase
FP:	Fluticasone propionate
GCH:	Goblet cell hyperplasia
GILZ:	Glucocorticoid-induced leucine zipper
GR:	Glucocorticoid receptor
GRE:	Glucocorticoid response element
ICAM:	Intercellular adhesion molecule
ICS:	Inhaled corticosteroid
iNOS:	Inducible nitric oxide synthase
IP-10:	IFN-γ–inducible protein 10
ITAC:	IFN-inducible T-cell chemoattractant
JNK:	c-Jun N-terminal kinase
MAPK:	Mitogen-activated protein kinase
MCC:	Mucociliary clearance
MCP:	Monocyte chemoattractant protein
Mig:	Monokine induced by IFN-γ
MIP-3α:	Macrophage inflammatory protein 3α
MKP-1:	Mitogen-activated protein kinase phosphatase 1
NF-κB:	Nuclear factor-κB
nGRE:	Negative glucocorticoid response element
NO:	Nitric oxide
PNEC:	Pulmonary neuroendocrine cell
PRR:	Pattern recognition receptor
SAPK:	Stress-activated protein kinase
STAT:	Signal transducer and activation of transcription
TLR:	Toll-like receptor
TSLP:	Thymic stromal lymphopoietin
UTR:	Untranslated region

EPITHELIAL RESPONSES IN ALLERGIC AIRWAYS AND IN THE ANTI-INFLAMMATORY ACTION OF GLUCOCORTICOID

The airway epithelium is a multifunctional, highly organized cellular layer separating the host tissue from the atmosphere. It provides many crucial homeostatic

From the Division of Allergy and Clinical Immunology, Johns Hopkins University.

C.S. is supported by National Institutes of Health grant R01 AI060990-01A1.
Disclosure of potential conflict of interest: The author has declared that she has no conflict of interest.
Received for publication September 18, 2007; revised October 26, 2007; accepted for publication October 29, 2007.

Reprint requests: Cristiana Stellato, MD, PhD, Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Circle, Room 1A.12A, Baltimore, MD 21224. E-mail: stellato@jhmi.edu.

0091-6749/$32.00

doi:10.1016/j.jaci.2007.10.041

respiratory functions, such as gas and fluid transport, oxidant defense, mucociliary clearance, and innate antimicrobial defense. At the same time, it is able to respond to either external factors brought by inhalation, such as viruses, pollutants, and allergens, or to molecular signals from neighboring or infiltrating cells, such as cytokines, chemokines, or lipid mediators, by producing a wide array of mediators contributing to key effector functions, such as inflammatory and immune responses, proliferation, and healing, ultimately to preserve the integrity of the airway mucosa.[1]

The involvement of epithelial responses in the pathogenesis of inflammatory and allergic diseases of the airways—such as asthma and rhinitis—is now a well established paradigm. The identification of pattern recognition receptors (PRRs), recognizing conserved components of microbial organisms, and their downstream signaling pathways provide molecular clues to the key participation of the epithelial layer in innate immune responses. Similarly, the synthesis of a host of proinflammatory and immunomodulatory molecules documented *in vitro* and *in vivo* in response to inflammatory stimuli—such as lipid mediators, growth factors, adhesion molecules, catabolic enzymes and enzyme inhibitors, and a wide array of cytokines, chemokines, and their receptors—clearly indicate that epithelial cells have a central role in mounting and amplifying the adaptive immune response. In particular, epithelial cells are key controllers of the recruitment and activation of inflammatory cells within the respiratory mucosa, a response that becomes pathogenic in the context of chronic allergic inflammation.

Mounting evidence suggests that besides the key participation in the adaptive response and in the maintenance of chronic mucosal inflammation, the airway epithelium displays a host of altered functions, such as an aberrant innate response to viral infection, alteration of barrier function, and defective wound repair. These features are now viewed as primary pathophysiological factors in asthma, especially in the more severe phenotype of the disease.[2-4] In support of this view, a number of genes identified as linked to asthma susceptibility, such as *dipeptidyl peptidase 10* and *G protein–coupled receptor for asthma susceptibility*, are uniquely expressed in terminally differentiated bronchial epithelium, suggesting that their function, although yet unknown, may be related to the maintenance of the epithelial barrier.[5]

The therapeutic role of glucocorticoids in allergic diseases is chiefly attributable to their powerful antiinflammatory properties, which have been further exploited in the treatment of airway allergic diseases by the development of inhaled corticosteroids (ICSs), topically active synthetic molecules with far fewer side effects than those carried by orally administered glucocorticoids. Central among the many mechanisms of the anti-inflammatory properties of glucocorticoids is the ability to blunt the infiltration of inflammatory cells within the affected tissue, which has been well documented in disease as well as in experimental models (see review[6,7]). Importantly, recent analyses of mechanisms of glucocorticoid action in airway epithelium have highlighted a concomitant protective action of glucocorticoids on innate immune responses that could be equally relevant in the ability of glucocorticoid therapy to diminish the occurrence of exacerbations brought by respiratory infections[8,9] (Fig 1).

The goal of this review is to summarize *in vitro* and *in vivo* studies demonstrating the effect of glucocorticoid therapy on those immune responses of the epithelial cell particularly relevant to allergic diseases. We then focus on more basic studies on the effects of glucocorticoids on airway epithelium by reviewing the changes in gene expression underlying glucocorticoid action and the molecular mechanisms mediating these events. At the end, we highlight emerging molecules and regulatory pathways that could be relevant in defining the specific response of the epithelium to glucocorticoid therapy. We refer (Fig 1) to *epithelial innate responses* as those involving molecules that function in host defense; and *epithelial adaptive responses* as those conveyed by molecules that principally function to amplify or modulate host immune function, both adaptive (eg, proinflammatory genes and mediators) and innate (eg, mucus hypersecretion, epithelial proliferation, and wound repair/remodeling).

PHENOTYPES AND FUNCTIONS OF NORMAL AND ASTHMATIC AIRWAY EPITHELIAL CELLS: COMPLEXITY OF GLUCOCORTICOID EFFECT ON THE AIRWAY EPITHELIAL LAYER

Action of glucocorticoids on the different epithelial phenotypes

In nondiseased state, the respiratory epithelium is composed of cellular components of different origin and phenotypes, whose discrete roles integrate in performing homeostatic and inducible functions. Embryologically, the airway epithelium derives from the endodermal layer of the embryo; histologically, it presents in the upper airways as a pseudostratified columnar layer containing basal, ciliated, and mucus-producing goblet cells, transitioning in the bronchioli into a cuboidal, single cell layer interspersed with secretory Clara cells, which is replaced in the lung alveoli by a squamous monolayer of type I and type II alveolar epithelial cells. From a structural and functional point of view, airway epithelial cells have been categorized in basal cells, ciliated cells, and nonciliated cells with secretory functions.[1]

The basal cells, firmly attached to the basement membrane of all but the very distal part of the airways, are a very important part of the epithelial-mesenchymal trophic unit of the airways. They function as primary progenitors of the ciliated and mucus-secreting cells and are highly metabolically active, producing 15-lypooxygenase products, cytokines, and endopeptidases.[1] Basal cells are important for the attachment to the basement membrane of the columnar epithelium and for many epithelial functions, from regulation of inflammatory response to protection from oxidative damage and to the transepithelial transport of water and fluids.[10] High levels of the cyclin-

dependent kinase inhibitor $p21^{waf}$ in basal cells of patients with severe asthma, but not in patients with mild asthma or healthy subjects, suggest their involvement in an antiapoptotic response in epithelial repair.[11]

The columnar ciliated cells, which account for more than 50% of the epithelial layer of the proximal airways, play a key role in innate immune host defense by carrying out mucociliary clearance and by responding, through multiple Toll-like receptor (TLR)–mediated and non–TLR-mediated pathways, to a host of pathogens. Columnar epithelium is the selective target of airway rhinovirus infections and is a potent producer of a host of proinflammatory mediators secreted in response to viruses and many other inflammatory stimuli.[12] The vast majority of such cellular responses are affected by glucocorticoids through selective, often stimulus-specific mechanisms.

Among the cells with secretory functions, goblet cells chiefly contribute to mucociliary clearance through production of mucins, and alteration of their number and secretory pattern are key pathophysiological features of asthma and other inflammatory airway diseases (see review[13,14]). Goblet cell function is affected by glucocorticoids both directly and indirectly as part of the complex control exerted by glucocorticoids on airway hypersecretion.

Clara cells are secretory epithelial cells responsible for the production of bronchiolar surfactant; they also retain stem cell potential in the lower airways, where basal cells are scarce.[15] In inflammatory settings, Clara cells can secrete cytokines in response to LPS stimulation[12] and undergo metaplastic transformation by differentiating into goblet cells, contributing to the goblet cell hyperplasia (GCH).[16] These cells can undergo profound phenotypic changes during inflammation, such as the selective induction of the *acidic mammalian chitinase* gene.[17] Suggestive of an effect of glucocorticoids on this pathway, the increase in mouse bronchoalveolar lavage levels of *acidic mammalian chitinase* induced by ovalbumin challenge was inhibited by treatment with dexamethasone.[18] Accounting for the role of endogenous glucocorticoid in regulating airway surface tension since the fetal stages of lung development, glucocorticoids upregulate in Clara cells the synthesis of phospholipids and surfactant proteins C and D.[19] Besides being key components of the pulmonary surfactant liquid, surfactant proteins C and D also play a role in innate immune responses.[20]

Pulmonary neuroendocrine cells (PNECs) are secretory, highly innervated cells dispersed within the epithelial layer, mostly in organized clusters defined as neuroepithelial bodies.[21] They contain, stored in secretory granules, several amines and neuropeptides such as serotonin, bombesin-like peptide, calcitonin gene-related peptide, substance P, and others. These cells exert complex functions throughout lung development and in the adult stage, including oxygen-sensing chemoreception, epithelial regeneration, and neuroendocrine function (see review[22]). Recently, PNEC-derived bombesin-like peptide was shown to target mast cells and mediate lung injury in baboon models of bronchopulmonary dysplasia, indicating a possible role of the pulmonary NE system in innate immunity.[23] Very little is known about glucocorticoid control of PNECs and their immune functions. Antenatal exposure to glucocorticoids increased the number of neuroepithelial bodies in animals,[24] whereas calcitonin secretion was inhibited in normal but not in neoplastic PNECs.[25]

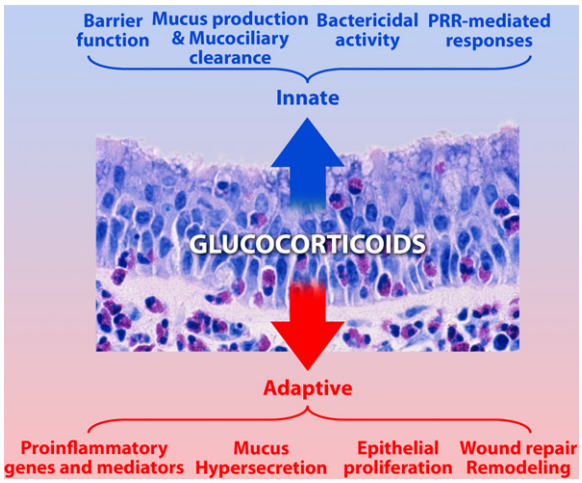

FIG 1. The immune response of airway epithelium as target of glucocorticoid therapy. The photographic *inset* shows the pseudostratified airway epithelium of a nasal polyp, with submucosal and intraepithelial eosinophilic infiltration (reprinted with permission from Beck LA, Stellato C, Beall LD, Schall TJ, Leopold D, Bickel CA, et al. Detection of the chemokine RANTES and endothelial adhesion molecules in nasal polyps. J Allergy Clin Immunol 1996;98:766-80.[176]). The epithelial layer of the respiratory system is formed by different cell phenotypes that integrate in providing important immune functions. Residing at the interface between the tissue and the external environment, airway epithelial cells perform multiple functions related to both innate and adaptive immune responses. These cells are primary targets of ICSs and of their anti-inflammatory action, which is mediated by coordinate modulation of both arms of the immune response.

Altered phenotype of epithelial cells in asthma: differential sensitivity to glucocorticoids

Examination of bronchial biopsies by electron microscopy combined with *ex vivo* studies using cultured primary epithelial cells have clearly established that airway epithelium displays a wide range of structural damage and functional alterations throughout the different stages of asthma.[26] Assessment of the amount and type of epithelial damage, however, has been hampered at the level of gross anatomy by a degree of shedding caused by the biopsy procedure itself. The occurrence of GCH with metaplasia of Clara cells is already detectable in the early phase of disease.[27] The damage to the ciliated cells is complex, including weakened attachment to the basal lamina,[28,29] damage to the cilia,[30] and cell shedding, detectable either in the sputum as cellular clumps (Creola bodies) or in bronchial biopsies, particularly in moderate chronic types of asthma. Airway epithelial cells of patients with asthma are more susceptible to oxidative stress–driven apoptosis[31] and display an activated phenotype with constitutively

active transcription factors (nuclear factor-κB [NF-κB], activator protein 1 [AP-1], signal transducer and activation of transcription [STAT]–1, STAT-6), and increased production of cytokines, growth factors, and other proinflammatory mediators (see reviewed[1]). In severe asthma, the balance between epithelial cell proliferation and apoptosis is profoundly altered. Epithelial desquamation is replaced by an increase in epithelium thickness caused by increased cell proliferation, although data on markers of epithelial apoptosis and cell survival are dissimilar.[32,33] These components of the epithelial repair process, activated in response to cell damage and activation, have been identified as key contributing factors for airway remodeling.[32,33] In fact, epithelial damage appears to correlate with bronchial hyperreactivity,[34] and increased thickening of epithelium and of the lamina reticularis is inversely correlated with baseline lung function (expressed as FEV_1%) in patients with severe asthma.[33] These morphological changes are also mirrored at molecular level in *in vitro* cultured asthmatic epithelial cells, which display increased/altered expression of transcription factors, heat shock proteins, proinflammatory mediators, adhesion molecules, collagen deposition, and several markers of cell proliferation and survival.[32,35,36] Alteration of important regulatory molecules for cell proliferation, such as the cyclin-dependent kinase inhibitor $p21^{waf}$ and—in patients with severe asthma—the epithelial growth factor receptor (EGFR) expression and activation state, are underlying the altered pattern of epithelial repair seen in severe asthma.[11,37,38] Given the persistence of discrete phenotypic changes in cultured asthmatic epithelial cells, and the absence of similar epithelial damage in other inflammatory disorders like bronchitis, cystic fibrosis, and chronic obstructive pulmonary disease, an abnormal epithelial response is now seen as an intrinsic factor in the pathophysiology of severe asthma.[3] Along with this primary defect, chronic exposure to allergens, viral infections, stress stimuli, and inflammatory mediators participates in determining structural and functional changes in the epithelial layer of susceptible individuals, sustaining the chronic inflammation and triggering the activation of an abnormal repair response.

How much of this altered epithelial phenotype is targeted by glucocorticoids? Evaluation of bronchial biopsies before and after glucocorticoid treatment of different lengths and courses of administration has clearly shown that glucocorticoids promote, to various degrees, restoration of the epithelial integrity in mild to moderate asthma. An increase in the number of ciliated cells, with restoration of the ciliated/goblet cell ratio, was documented by electron microscopy after a 3-month treatment with ICSs in newly diagnosed patients with asthma,[39] and epithelial cells in sputum decreased after glucocorticoid treatment.[40] In a small study, glucocorticoid treatment had a positive effect on ciliogenesis.[41] However, only a partial reduction of epithelial damage was seen after 10 years of daily ICS treatment in patients with severe asthma.[42]

The mechanism behind glucocorticoid restoration of epithelium integrity is just as complex as the cause of epithelial distress. Clearly, the inhibition of expression of inflammatory and chemotactic genes from many cellular sources and the control of leukocyte infiltration by glucocorticoids largely prevents the damage to epithelium that is secondary to the cytokines and mediators secreted by infiltrating leukocytes. A key direct component of glucocorticoid action is the potent suppression by glucocorticoids of epithelial-derived cytokines and chemoattractants that promote the recruitment, survival, and activation of T cells, eosinophils, and other effector cells (see review[8,43]). This action blunts an important source of alteration of the epithelial phenotype caused by cytopathic or proinflammatory effects of mediators secreted by the recruited leukocytes.[44]

Furthermore, glucocorticoids support epithelial integrity by promoting the maintenance of proper cell-cell adhesion. Treatment of primary airway epithelial cells with dexamethasone potently reversed the TNF-α–mediated decrease of expression of E-cadherin, β-catenin, and γ-catenin, adhesion molecules important in the maintenance of proper intercellular junction.[45]

In contrast, as the epithelium of patients with severe asthma appears intrinsically different from milder forms of the disease, so does the response to glucocorticoids. Markers of epithelial activation and proliferation are persistently elevated in glucocorticoid-dependent patients with asthma[32,33]; glucocorticoid treatment *in vivo* and *in vitro* fails to inhibit the increase in expression and phosphorylation of the EGFR, and to inhibit EGFR-mediated IL-8 expression.[38] The increased expression of $p21^{waf}$ in the airway epithelium of patients with either mild or severe asthma is also specifically resistant to glucocorticoid treatment *in vivo* and *in vitro*, whereas other glucocorticoid responses are preserved.[11] A 2-week oral glucocorticoid regimen in patients with moderate to severe asthma led to inhibition of the epithelial expression of the profibrotic cytokines IL-11 and IL-17, but not of TGF-β and collagen deposition.[46] Overall, it appears that glucocorticoids have a marginal direct influence on the epithelial component of the remodeling process.

EFFECT OF GLUCOCORTICOIDS ON MUCUS PRODUCTION AND AIRWAY MUCOCILIARY CLEARANCE

Together with the cough reflex, mucociliary clearance (MCC) is an essential nonspecific mechanism of defense of the airways that is carried out by the ciliated cells and by goblet cells. Evaluation of bronchial specimens from asthmatic deaths[47] as well as bronchial biopsies from moderate and mild asthma[48] shows that the epithelial cell types involved in this function display various degrees of damage. Besides the alterations of ciliated cells,[30] there is hyperplasia of goblet cell as well as of submucosal glands, which leads to increased storage and degranulation of the main secreted mucins of the respiratory tract, whose protein backbones are encoded by the *MUC5AC* and *MUC5B* genes.[49] Mucus is also altered in composition

and viscosity by the presence of components of the inflammatory plasma exudate, by increased water and electrolyte passage in the airway lumen, and by increased cellular debris caused by local cell death.[13] Allergen challenge studies confirmed that factors driving the hypersecretory state are the inflammatory mediators released mainly from infiltrating $CD4^+$ T cells, as well as the alteration of epithelial fluid exchange and the release of neuropeptides from intraepithelial innervation. These events in turn affect the ciliary apparatus structurally and functionally (see review[13,50]). Because the efficacy of MCC relies on the integrity of the ciliary activity as well as on optimal physicochemical and rheologic characteristics of the mucus, these epithelial changes lead to various degrees of impairment of the MCC process and become a major cause of airway obstruction, a key pathophysiological event in asthma exacerbations and asthma deaths (see review[13,51]).

Comparison of airway mucosa in biopsies of patients with asthma before and after glucocorticoid therapy shows an amelioration or normalization of this hypersecretory phenotype, with an increase in ciliated cells and restoration of the normal ratio of ciliated/goblet cells.[39] The mechanism of this effect is complex and not completely understood; it can be ascribed in part to indirect effects, such as the inhibition by glucocorticoids of the expression of inflammatory mediators that stimulate GCH and/or mucin production. Among these, T_H2 cell–derived cytokines such as IL-4, IL-13, and IL-9 are major inducers of GCH, as well as the potent proinflammatory cytokines TNF-α, IL-1-β, and IL-6 (see review[14,49]). The inhibition of chemokine-driven recruitment of inflammatory cells[8,43] and the effect on microvascular leakage[52] also play important but indirect roles in the control of GCH by glucocorticoids.

The mechanisms controlling GCH, the expression of airway *MUC* genes, and the process of goblet cell degranulation are distinct,[14] and glucocorticoids differentially affect these events in a direct fashion. In a model of ovalbumin-induced lung inflammation, systemic administration of dexamethasone (at 1 μg/kg/d) for 8 days, in parallel with the allergen challenge, reduced the increase in goblet cell numbers seen in sham-treated animals, inhibited inflammatory cell recruitment, and significantly reduced the severity of established GCH when administered postchallenge.[53] However, a shorter (3-day) treatment regimen in the same mouse model did not affect GCH.[54] Dexamethasone inhibited *MUC5AC* expression in human and rodent primary epithelial cells and in the human cell lines A549 and NCI-H292, but it did not affect its secretion in rodent primary cells.[55-57] In contrast, a 6-day administration of 0.5 mg/kg dexamethasone failed to reduce *MUC5AC* overexpression and GCH in a mouse model of IL-13 overexpression while fully inhibiting CCL11/eotaxin expression and eosinophil accumulation in the lung.[55] Importantly, IL-13–dependent hypersecretory changes were found to be STAT-6–dependent[58]; in airway epithelial cells, both the expression and the activation of STAT-6 induced by IL-4 are not sensitive to glucocorticoid action.[59] In human asthma, Groneberg et al[48] found no difference in the expression and distribution of *MUC5AC* and *MUC5B* in bronchial biopsies from patients with mild asthma after a 1-month period of daily treatment with 1600 μg of the ICS budesonide.

Mucus cell metaplasia has also been associated with the overexpression of a $Na^+/K^+/Cl^-$ cotransporter, a regulator of transepithelial Cl secretion, and of a member of the $Ca2^+$-activated Cl^- channel family, although the function of the latter molecule as Cl^- channel is still controversial.[60-62] The effect of glucocorticoids on these molecules is not known; however, some of the antisecretory effects of glucocorticoids could be mediated by the inhibition of Cl^- secretion occurring through rapid, nongenomic mechanisms documented in human bronchial epithelial cells *in vitro* within 15 minutes of exposure to low concentrations of dexamethasone.[63]

Studies looking at MCC in an allergen challenge sheep model and in patients with mild asthma showed that short-term courses of ICS and systemic glucocorticoids affected MCC only transiently, or did not have any effect, respectively, despite improvement of patients' FEV_1 in the latter study.[64,65] However, an earlier study reported a significant improvement of MCC in outpatient, steroid-responsive patients with asthma after a 4-week treatment with systemic glucocorticoids.[66]

Together, these data suggest that glucocorticoids can directly affect goblet cells by modulating mucin gene expression, but such an inhibitory effect appears to be time-dependent, concentration-dependent, and stimulus-dependent. The lack of effect of short-term glucocorticoid treatment found by *in vivo* studies suggests that the global anti-inflammatory effects of glucocorticoids on the mediators and processes driving hypersecretory changes, over the course of chronic treatment, may chiefly dictate the drug's ability to normalize GCH and mucus hypersecretion, allowing the epithelium to regain an efficient MCC function through restoration of its normal structure.

New insights on the pathways involved in mucus hypersecretion warrant more studies on the effects of glucocorticoids on goblet cells. Activation of the EGFR induces *MUC5AC* expression and goblet cell metaplasia, an event inhibited by specific EGFR tyrosine kinase inhibition.[67] The specific effect of glucocorticoids on EGFR-mediated mucin production is not known; however, the increased epithelial expression or activation of EGFR in patients with mild or severe asthma was not modified by glucocorticoid treatment *in vivo* or *in vitro*.[37,38] Given the potential relevance of the EGFR signaling pathway in regulating mucin expression in innate immune responses,[68] this lack of direct glucocorticoid effect may be consistent with the hypothesis of a sparing effect of glucocorticoids on innate immunity[8]; on the other hand, it constitutes one of the most important glucocorticoid-insensitive epithelial pathways in severe asthma.[3,38] Given the insensitivity to glucocorticoids of IL-13–induced GCH and of STAT-6 expression and activation in airway epithelium,[55,59] it can be hypothesized that glucocorticoids would not affect the IL-13–dependent and STAT-6–dependent expression of the SAM pointed domain-containing ETS transcription

factor, recently identified in mouse airway epithelium in association with GCH.[69]

EFFECTS OF GLUCOCORTICOID ON EPITHELIAL INNATE IMMUNE RESPONSES

Standing at the edge of the respiratory tree and facing the outside world, epithelial cells are the prime protagonist of the innate immune surveillance in the airways. Once past the mucus barrier, a large host of innate antimicrobial responses are carried by epithelial cells on cell activation mediated by transmembrane and cytoplasmic PRRs, belonging to either TLR and non-TLR families, or other entry receptors recognizing a wide spectrum of microbial components and proteins, named collectively *pathogen-associated molecular patterns*.[70]

The epithelial response that follows pathogen recognition by PRRs is complex and includes increased expression of molecules that function in host defense such as cytokines, chemokines, adhesion pathways, enzymes, and compounds with antimicrobial properties. Comprehensive reviews of the receptors, pathways, and mediators used by epithelial cells to mediate their innate immune response have been published recently.[8,71,72] The innate immune response has been found to be profoundly impaired in the epithelium of patients with asthma. After infection with rhinovirus, asthmatic epithelium displays a deficiency in production of IFN type I and a deficiency in induction of apoptosis, permitting infected cells to live longer and shed more viruses.[73,74] Aberrant innate epithelial responses are thought to be a primary factor of asthma pathophysiology, together with a viral and allergic component, because research by Holtzman et al[2] indicates that a subset of genes triggered by the innate response of epithelial cells to fight viral infections (STAT-1, intercellular adhesion molecule [ICAM]–1, CCL5/RANTES, IL-12p40) appears to be also aberrantly expressed in asthma, and to be only partially sensitive to glucocorticoids. Overall, the response elicited by viral infection is less glucocorticoid-sensitive in the asthmatic epithelium. Treatment with high doses of glucocorticoids does not rescue the diminished expression of IFN-β induced by rhinovirus *in vitro* in ICS-naive epithelial cells obtained by bronchial brushing from patients with mild asthma, whereas double-stranded RNA-induced upregulation of IFN-β is significantly inhibited in cultured primary epithelial cells and in 2 epithelial cell lines by treatment with the potent topical glucocorticoid fluticasone propionate (FP).[9] Epithelial expression of ICAM-1 is profoundly inhibited *in vitro* in normal primary cells or cell lines.[75,76] However, a 2-week regimen of the ICS budesonide in steroid-naive patients with mild asthma did not inhibit the increase in epithelial ICAM expression induced by an experimental infection with rhinovirus 16, as assessed by immunohistochemical evaluation of bronchial biopsies.[77] Collectively, these data suggest that some features of the epithelial phenotype in asthma are selectively resistant to glucocorticoids.

The major culprit of exacerbations of asthma and other chronic allergic airway diseases are indeed microbial infections, in particular those driven by viruses.[78] The cytokines and mediators, generated in response to microbial invasion by the epithelium and by the recruited inflammatory cells, activate the hypothalamic-pituitary axis. This event produces a rise in the endogenous corticosteroids from the adrenal cortex, which limits the overexpression of inflammatory responses.[79] Because regular ICS therapy consistently decreases the overall frequency of disease exacerbations (see review[80]) without favoring susceptibility to respiratory infections, it is relevant to understand the mechanism of action of glucocorticoids on the pathways of innate immunity. Mounting data indicate that glucocorticoids have a global protective effect on innate immunity through an enhanced expression of genes involved in host defense.[81-83] Regarding the epithelial innate immune response, Schleimer[8] proposes that although glucocorticoids are potent inhibitors of epithelial-derived adaptive proinflammatory responses, they spare or enhance the expression of epithelial genes involved in innate immunity. A recent proof-of-concept study by Zhang et al[9] shows that the TLR-3–mediated epithelial expression of many molecules with host defense functions was either unchanged or increased by cell cotreatment with FP. At the same time, TLR-3–induced expression of inflammatory cytokines and chemokines was, as expected, significantly inhibited by FP. Furthermore, they identify the transcription factor CCAAT/enhancer binding protein β as a selective mediator of glucocorticoid action on molecules involved in host defense. Combined cell treatment with FP and TLR-3 ligands induced the epithelial expression and function of CCAAT/enhancer binding protein β, and silencing of this transcription factor significantly reduced the induction of host defense genes without affecting the concomitant inhibitory activity of FP on inflammatory cytokines and chemokines.[9] Some of the effects of glucocorticoids on host defense molecules reported in this study are summarized in Table I.

Another process used by glucocorticoids to boost epithelial innate immune responses is the upregulation of TLR-2, which has been reported to occur *in vitro* in synergism with either bacterial products or with inflammatory cytokines[84-87] and to mediate an increased production of cytokines and chemokines.[84,87] TLR-2 recognizes several products of Gram-positive bacteria and is upregulated by TNF-α through an NF-κB–mediated pathway.[88] TLR-2 is also expressed in the adrenal tissue, and TLR-2 deficiency is associated with an impaired glucocorticoid response.[89] The molecular mechanism of glucocorticoid-induced TLR-2 in epithelium consists of distinct mechanisms, including induction of enzymes that act on negative regulators of the TLR pathways or TLR-driven transcriptional regulation (see review[90]). Glucocorticoids were found synergistically to enhance TLR-2 expression induced by nontypeable *Haemophilus influenzae* in primary epithelial cells through a selective upregulation of the mitogen-activated protein kinase (MAPK) phosphatase 1 (MKP-1), which inactivated p38 MAPK, a negative

regulator for TLR-2 expression.[84,85] Glucocorticoid-induced potentiation of epithelial TLR-2 is also triggered by IL-1β.[84,85] In this case, in addition to p38, glucocorticoid-induced MKP-1 inactivated the c-Jun N-terminal kinase (JNK), which also was found to be a negative regulator of TLR-2 expression.

Coexisting with the negative cross-talk between glucocorticoid-induced MKP-1 and MAPKs, Hermoso et al[86] demonstrated that upregulation of TLR-2 in A549 lung epithelial cells by dexamethasone is also a result of a complex control exerted at transcriptional level by a cooperative interaction between NF-κB and STAT, each engaging their respective consensus sequences on the TLR-2 promoter and recruiting the glucocorticoid receptor (GR), through its activation function domain 1, to a glucocorticoid response element–like element present in the 3′ end of the TLR-2 promoter.

EFFECTS OF GLUCOCORTICOIDS ON EPITHELIAL FUNCTIONS INVOLVED IN ADAPTIVE IMMUNE RESPONSES

After the triggering of a direct, innate immune response, pathogen recognition elicits a shift toward higher adaptive immune responses, and epithelial cells actively participate in this process. In response to PRR engagement, epithelial cells secrete factors that recruit and activate dendritic cells (DCs) as well as leukocytes, serving as an essential bridge between innate and adaptive responses; subsequently, the epithelium is able to respond to the cytokine milieu established by the recruited inflammatory cells with the expression of discrete patterns of chemokines, cytokines, growth factors, and inflammatory mediators that maintain and amplify an inflammatory loop established between T_H2-driven epithelial activation and selective, epithelial-driven leukocyte recruitment.[8,43]

Effect of glucocorticoids on the cross-talk of epithelium-DCs

The proximity of DC and epithelium within the airway mucosa and the wide range of mediators with potential reciprocal regulation make the epithelial-DC interaction a critical component of the airway response to antigens,[91] although most of these interactions are still ill-defined. Activation of epithelial cells by allergen, bacterial proteins, and cytokines leads to the upregulation of ICAM-1, GM-CSF, and chemokines such as CCL20/macrophage inflammatory protein 3α (MIP-3α), CCL5/RANTES, and CXCL10/IFN-γ–inducible protein 10 (IP-10) shown to influence the recruitment and/or maturation of DC precursors within the airway mucosa.[92,93] Moreover, a variety of TLR ligands, T_H2-derived cytokines, and rhinovirus infection induce *in vitro* in airway epithelium the production of the IL-7–like cytokine thymic stromal lymphopoietin (TSLP).[94-96] TSLP induces $CD11c^+$ DCs to drive naive T_H cells toward a T_H2 phenotype via the OX40-OX40L pathway.[97] The expression of TSLP is increased *in vivo* in the epithelium of patients with asthma compared with healthy subjects, and it correlates with disease severity and with the expression of T_H2-recruiting cytokines.[98] The expression of TSLP is therefore a critical link between the initial epithelial response and the establishment and maintenance of the allergic response in the airways, elicited both in the setting of innate and adaptive responses, and clearly represents an important therapeutic target.[4] Other epithelial-derived products such as β-defensin-2, potent chemoattractant and activator of immature DC, TGF-β, and prostaglandin E_2[99] have the potential to modulate DC function as well.

Glucocorticoids negatively regulate the epithelial expression of many of these molecules, such as GM-CSF, which acts on DCs as a potent growth factor and activator,[100,101] TSLP,[96] and β-defensins.[9] These data indicate that inhibition of epithelial-derived signals enabling the recruitment and activation of DCs is an important component of the anti-inflammatory effect of glucocorticoids. However, the expression of CCL20/MIP-3α, another potent chemoattractant for immature DCs and CCR6 ligand, is increased by glucocorticoid treatment *in vitro* in primary epithelial cells.[9] Given the potent antibacterial function of CCL20/MIP-3α, the authors of this study hypothesize that such regulation favors the protective effect of glucocorticoids on host defense responses. Further clarification of

TABLE I. Glucocorticoid regulation of the expression of molecules involved in host defense in human primary bronchial epithelial cells*

Primary bronchial epithelial cells: host defense molecules	Basal expression or induction (+) by treatment with dsRNA	Response to glucocorticoid in dsRNA-treated cells†
Complement		
C3	+	Enhanced
Factor B	+	Preserved
Defensins		
HBD-1	+	Preserved
Collectins		
MBL	Basal	Preserved
SpD	+	Preserved
Pentraxins		
CRP	−	Induced
Other antimicrobial proteins		
Lysozyme	Basal	Preserved
Lactoferrin	Basal	Preserved
SLPI	Basal	Preserved
SAA	+	Preserved
CCL20/MIP3-α	+	Preserved

Based on Zhang N, Truong-Tran QA, Tancowny B, Harris KE, Schleimer RP. Glucocorticoids enhance or spare innate immunity: effects in airway epithelium are mediated by CCAAT/enhancer binding proteins. J Immunol 2007;179:578-89.[9]

*Gene expression is defined as basal when detected in unstimulated cells and unchanged by treatment; (+) indicates significant stimulus-dependent upregulation; (−) indicates lack of expression in resting and stimulated samples in the absence of glucocorticoids.

†Glucocorticoid responses were significantly increased (enhanced) or spared (preserved).

the pathways and molecules responsible for DC accumulation in the lungs will be necessary to establish the relevance of glucocorticoid-regulated changes in epithelial-derived DC chemoattractants.

Effect of glucocorticoids on epithelial-driven leukocyte recruitment and activation

The inhibition of leukocyte infiltration is a key component of the anti-inflammatory action of glucocorticoids, and of the control of the severity of asthma and other allergic airway diseases.[102] It is well established that this particular effect of glucocorticoids is achieved in large part by inhibiting the expression of cytokines, chemokines, and inflammatory mediators promoting the recruitment, proliferation, activation, and survival of inflammatory cells such as T_H2 cells, eosinophils, and basophils.[102] Airway epithelial cells are a key source of such factors, and inhibition of epithelial gene expression relevant to leukocyte recruitment and activation is indeed a major target of therapy with ICSs.[8,43] Blockade of airway eosinophilic infiltration by glucocorticoid treatment relies largely on the inhibition—observed both *in vitro* and *in vivo*—of epithelial-derived GM-CSF, which promotes eosinophil survival, and of the expression of eosinophilic chemokines and CCR3 ligands such as CCL11/eotaxin-1, CCL24/eotaxin-2, and CCL13/monocyte chemoattractant protein (MCP)–4 (see review[43]). Inhibition of T_H2 cell recruitment relies as well on the glucocorticoid inhibition of epithelial CCR4 ligands such as CCL17/thymus- and activation-regulated chemokine.[103] Importantly, overexpression of both eosinophilic and T_H2 cell–specific chemokines is mostly under the joined control of TNF-α and of T_H2-derived cytokines, like IL-4 and IL-13.[103,104] Therefore, glucocorticoid inhibition of epithelial CCR3 and CCR4 ligands blunts an important inflammatory loop between epithelial-activating signals from T cells and T cell–recruiting signals from epithelium.

Similarly, when activated by IFN-γ in the context of a T_H1-driven response, epithelial response is relatively skewed toward the recruitment of CXCR1-bearing neutrophils and CXCR3-bearing T_H1 cells through the induction of the appropriate ligands, CXCL8/IL-8, CXCL1/growth-regulated oncogene α, CXCL5/epithelial neutrophil activating peptide-78, CXCL9/monokine induced by IFN-γ (Mig), CXCL10/IP-10, and CXCL11/IFN-inducible T-cell chemoattractant (ITAC), as well as of potent monocyte chemoattractants like CCL5/RANTES (see review[8]). The CXCR3 ligand chemokines are upregulated during viral responses[105] and in T_H1-driven diseases such as tuberculosis.[106] Although the production of CCL5/RANTES is significantly inhibited by glucocorticoid both *in vitro* and *in vivo*,[107,108] expression of the IFN-γ–induced CXCR3 ligands CXCL9/Mig, CXCL10/IP-10, and CXCL11/ITAC, which are possibly the most abundant chemokines secreted by epithelium, were found to be insensitive to dexamethasone in epithelial cell lines as well as in primary cells,[106] although a later *in vitro* study found CXCL10 to be suppressed in primary epithelial cells by the more potent glucocorticoid fluticasone.[109] Similarly, the inhibition of CXCL8/IL-8 by glucocorticoid treatment has been reported *in vitro*[9,109] and in bronchial biopsies of patients with mild asthma,[110] but other studies found unaltered, or even enhanced, expression of CXCL8/IL-8 and CXCL10/IP-10 after glucocorticoid treatment in bronchial biopsies from patients with moderate-to-severe asthma.[38,111] The neutrophilic CXCL5/ENA-78 was enhanced as well by treatment with fluticasone.[109] Clearly more studies are needed to establish the glucocorticoid sensitivity of CXC chemokine expression according to the trigger, severity, and treatment regimen of asthma. It is possible that a relative lack of glucocorticoid sensitivity of key neutrophilic chemokines may have a permissive role for the persistent neutrophilia observed in more severe asthma and during acute viral infections, which are clinical settings with a known limited response to glucocorticoids. Similarly, lack of suppression of CXCR3 ligands may underline the coexistence of a T_H1-driven component in mucosal inflammation after viral infection.[2]

The mechanisms regulating glucocorticoid action on epithelial-derived chemokines entail both transcriptional and posttranscriptional regulation[43,112]; however, the molecular basis of the unresponsiveness to glucocorticoids of some CXC chemokines in epithelium are yet to be uncovered.

Effect of glucocorticoids on cytokine-inducible enzymes: COX-2 and inducible nitric oxide synthase

Epithelial cells express 5-lipooxygenase, 2-lipooxygenase, and 15-lipooxygenase and inducible COX enzymes, enabling them to generate lipid mediators that influence vascular and smooth muscle tone.[113] Inhibition of COX-2 by glucocorticoids in airway epithelium has been described *in vitro* and *in vivo*.[114,115] In the epithelial cell line BEAS-2B, the inhibitory effect of glucocorticoid on COX-2 was found to be specific, because neither COX-1 nor the constitutive and inducible forms of phospholipase 2 were affected, and a generation of prostaglandin E_2 induced by bradykinin was inhibited by cell treatment with fluticasone, budesonide, or triamcinolone in this rank order of potency.[116]

Endogenous nitric oxide (NO) is a physiological component of exhaled air. Levels of exhaled NO are increased in patients with asthma by *de novo* synthesis, through the activation of the inducible form of the NO synthase (iNOS), which is highly expressed in airway epithelium.[117] The upregulation of iNOS in the epithelium of patients with asthma, as well as the levels of exhaled NO, are controlled by ICS therapy.[115] However, the ultimate proinflammatory or anti-inflammatory role of this pathway—and therefore the relevance of its control by glucocorticoids—is not fully established yet.[115]

Table II summarizes the results of several *in vitro* and *in vivo* studies showing glucocorticoid modulation—at the mRNA and/or protein level—of the main epithelial products acting on recruitment and activation of inflammatory cells (see review[8,43]).

MOLECULAR DETERMINANTS OF GLUCOCORTICOID ACTION IN AIRWAY EPITHELIUM

Integrated mechanisms of action for a multitasking hormone

Glucocorticoids exert their anti-inflammatory action through a global, integrated action on the mechanisms that regulate gene expression, from early signaling events to the nuclear, transcriptional mechanisms and to posttranscriptional and posttranslational regulatory events occurring mainly in the cytoplasm. The study of glucocorticoid action in genome-wide studies has clearly shown that the net result of this complex regulation, which acts in a highly context-specific, cell-specific, and gene-specific fashion, is a global change in gene expression patterns that coordinately affect different aspects of immune responses.[81,82] Many recent and thorough reviews have critically analyzed the current knowledge on established and emerging mechanisms of the anti-inflammatory action of glucocorticoids.[8,90,118-122] In this review, we briefly refer to the main areas of gene regulation affected by glucocorticoids and then focus in particular on pathways of glucocorticoid regulation that are emerging as potentially relevant for therapeutic modulation of the airway epithelial response.

The influence of glucocorticoids on gene expression is initiated by binding of endogenous or synthetic glucocorticoid ligands, which freely pass the cellular membrane, to the GR in the cell cytoplasm. Two main isoforms of the human GR are generated by alternative splicing of the GR transcript: the predominant isoform is GRα, which displays steroid-binding and transactivating activity and is present, in complex with chaperon proteins, within the cytoplasm of the majority of cell types. The isoform GRβ possess a shortened ligand binding domain and is expressed less ubiquitously and at far lower levels (see review[123]). Although GRβ has been found to be devoid of DNA-binding and transactivating activity and is functionally characterized as a dominant negative of GRα activity,[123] recent data identified the binding of a unique ligand, the glucocorticoid antagonist RU-486, to GRβ and the ability of this isoform to regulate gene expression in the absence of GRα.[124]

Ligand binding leads to phosphorylation and conformational changes of the GRα (from now on referred to as GR), which disassociates from a multimeric complex with heat shock proteins within the cytoplasm and translocates in the nucleus. Homodimers of ligand-activated GR act as a transcription factor that can influence gene transcription through DNA-dependent mechanisms by binding to a set of structurally and functionally diverse consensus sequences, the GRE, present within the 5′ promoter region of target genes.[125] The functional outcome—activation or repression—of DNA-dependent transcriptional regulation by GR is contingent on the type and sequence of each GRE, the presence of other cofactors interfacing with the GR through distinct regulatory domains, and the local conformation of the chromatin structure.[90] Studies in the last decades have shown, however, that transcriptional inhibition of proinflammatory genes by glucocorticoid can also be mediated by DNA-independent ''transrepression,'' mediated by interactions with other DNA-binding transcription factors such as AP-1 and members of the NF-κB family.[126-128] Other transcription factors, like members of the STAT and Forkhead box families, interact with glucocorticoids and modulate aspects of the immune response.[118,129] Downstream of transcription factor binding, glucocorticoids may also prevent gene transcription by altering chromatin structure, by either increasing histone deacetylation or decreasing histone acetylation, and by hindering the access of transcription factors to their response elements by causing DNA to remain bound to histones (see review[130]).

Positive transcriptional regulation or ''transactivation'' of gene expression is rapidly rising in ranking as a relevant mechanism in controlling the action of glucocorticoid on immune responses.[81,82] Some of the glucocorticoid-induced genes, which are discussed in the following paragraphs, mediate effects on MAPK-mediated signaling and posttranscriptional regulatory events, extending the mechanism of glucocorticoid control on gene expression to pathways of cell signaling and to control of mRNA turnover and translation.[122,131-133]

Glucocorticoids can also produce rapid nongenomic effects, which are defined as biological actions that precede or do not influence gene expression, and are mediated by the activation of signaling cascades.[134] The role of nongenomic mechanisms in the glucocorticoid therapy of asthma is still controversial and represents a growing area of investigation (see review[63,122]). Nongenomic effects appear to be particularly relevant for glucocorticoid action on endothelium.[135] In airway epithelium, nongenomic mechanisms regulate intracellular pH, Ca^{2+}, and protein kinase A activity and inhibit Cl^- secretion, potentially mediating some of the antisecretory effects of glucocorticoids.[63]

Glucocorticoid receptor in airway epithelium

Early *in vitro* studies demonstrated through Northern and Western blot analysis that primary airway epithelial cells as well as the cell line BEAS-2B express a functional GR, to which radiolabeled ligands bound with high affinity. In BEAS-2B cells, treatment with glucocorticoid-activated a GRE-mediated reporter activation, as well as the transrepression of AP-1 and NF-κB reporters, substantiating the hypothesis that epithelial cells were a target of the anti-inflammatory effects of ICSs.[136] Localization of GR in airway epithelium was detected in normal and asthmatic bronchial biopsies at the mRNA and protein levels.[137] A decreased GRα/GRβ ratio correlates with the development of glucocorticoid resistance in asthma and other inflammatory diseases.[123,138] A preferential increase of GRβ protein levels over GRα followed by a loss of glucocorticoid response was generated *in vitro* by treatment of HeLa cells with inflammatory cytokines, indicating a mechanism by which inflammation may

TABLE II. Effect of glucocorticoid *(GC)** on expression of epithelial-derived genes acting on recruitment, survival, and activation of inflammatory cells

Cytokines	GC	Growth factors/ receptors	GC	Chemokines	GC	Adhesion, enzymes, other	GC
				CXC			
IL-1β	↓	GM-CSF	↓	CXCL1/Gro-α	↓	ICAM	↓↔
IL-6	↓	TGF-β	↓	CXCL5/ENA-78	↑	Vascular cell adhesion molecule	↓
IL-9	↓			CXCL8/IL-8	↓↔↑	E-cadherin	↓
IL-11	↓	EGFR	↔	CXCL9/Mig	↔	β-Catenin	↓
IL-12gp120	↓			CXCL10/IP-10	↔↓	γ-Catenin	↓
IL-16	↓			CXCL11/ITAC	↔		
TNF-α	↓					iNOS	↓
				CC		COX2	↓
				CCL2/MCP-1	↓		
				CCL5/RANTES	↓	β-Defensin	↓
				CCL11/eotaxin-1	↓		
				CCL13/MCP-4	↓		
				CCL17/thymus- and activation-regulated chemokine	↓		
				CCL24/eotaxin-2	↓		

*Up, down, and bidirectional arrows denote, respectively, upregulation, inhibition, and no change described at mRNA and/or protein level after GC treatment in *in vitro* and *in vivo* studies (see reviews by Schleimer[8] and Stellato and Schleimer[43]). For some genes, different outcomes have been reported in different studies.

induce glucocorticoid resistance.[139] Although the correlative value of this finding with glucocorticoid resistance is established in several diseases treated with glucocorticoids, the pathophysiological relevance of this finding in glucocorticoid-resistant asthma is still controversial,[140-142] as is its occurrence in airway epithelium. Immunohistochemical studies on expression of GRβ in cases of severe or fatal asthma and nasal polyposis showed that increased protein levels of GRβ were mostly in $CD3^+$ T cells, eosinophils, and macrophages.[143,144] A subsequent study did find immunohistochemical evidence of a selective increase of GRβ in patients with severe asthma compared with patients with asthma with moderate disease.[145] However, an earlier study evaluating the expression of GR in bronchial epithelium in patients with asthma—and extended later by the same group to PBMCs—found no difference in GR expression among control subjects and glucocorticoid-responsive and glucocorticoid-dependent patients with asthma, despite the loss of glucocorticoid inhibition of GM-CSF expression found only in the epithelium of glucocorticoid-dependent patients with asthma.[36,140] More studies will be necessary to establish unequivocally whether the GRβ isoform is in fact predominant in epithelial cells of glucocorticoid-resistant patients with asthma, what level of GRβ overexpression is needed *in vivo* to tip cells over glucocorticoid resistance, and what are the mechanisms, beyond the action as dominant negative of GRα function, that are carried out by GRβ in the context of glucocorticoid-resistant response during inflammation, also in light of the recent data on specific transactivating properties of GRβ.[124]

Another layer of complexity in glucocorticoid regulation was added by the important discovery of multiple GRα isoforms arising from the GR gene by translational mechanisms.[146] These isoforms display tissue-specific patterns of distribution and only partially overlapping transcriptional profiles. The data suggest that the ratios of various GR isoforms in a given cell type can modify glucocorticoid transcriptional activity. It is likely that future studies will focus on the characterization of the profile of GRα isoforms expressed in airway epithelial cells, because this would greatly advance the understanding of epithelial-specific responses to glucocorticoids and their potential alterations in glucocorticoid resistance in asthma.

DNA-independent gene regulation in epithelium: beyond transrepression

Interaction of the GR with AP-1 and with members of the NF-κB family leading to repression of the transcriptional activation of proinflammatory genes is an established mechanism of the anti-inflammatory activity of glucocorticoids.[127,128] However, evidence of glucocorticoid-driven transrepression of AP-1–mediated or NF-κB–mediated regulation has been very discordant in epithelial cells, suggesting that this mechanism may occur according to discrete conditions of cell stimulation and the timing or concentration of the glucocorticoid. Epithelial immunostaining for activated NF-κB was inhibited by an 8-week treatment with budesonide in patients with mild asthma, in conjunction with inhibition of the NF-κB–driven genes GM-CSF, TNF-α, and vascular cell adhesion molecule 1[110]; similarly, a 6-week regimen with ICSs led to a reduction, observed by electromobility shift assay, of NF-κB binding to nuclear extracts obtained from bronchial biopsies of patients with mild asthma.[147] In contrast, a 4-week course of treatment with inhaled fluticasone did not influence epithelial NF-κB expression and DNA-binding activity.[148] *In vitro*, cytokine-induced NF-κB binding to the iNOS promoter

was inhibited by glucocorticoid treatment in A549 cells[149]; however, several studies found that NF-κB expression, its nuclear translocation, or its binding to DNA, which mediated the expression of cytokines and chemokines, was not modified by glucocorticoid treatment.[150-152] Interestingly, another key transcriptional activator of T_H2-skewed immune responses, STAT-6, is not a direct target of glucocorticoid action in epithelial cells.[59,152]

It is important to underscore that protein:protein interactions between GR and transcription factors do not occur only in the context of transrepression, but can also occur to implement cooperative transcriptional regulation between proinflammatory and anti-inflammatory signals. As in the case of activation function domain 1–dependent tethering of GR to DNA-bound NF-κB and STAT in inducing transactivation of the TLR-2 gene.[86] Along with this novel signaling pathway, highlighting the complex cross-talk between glucocorticoids and the innate immune responses, new insights obtained in mouse macrophages indicate that glucocorticoids interfere with TLR signaling also by competition of GR for binding with corepressor molecules like glucocorticoid receptor interacting protein 1, used by the transcription factor interferon regulatory factor 3 in mediating TLR-3/4 activation of several IFN and chemokine genes.[153] It will be of interest to ascertain whether this pathway is targeted by glucocorticoids in epithelium as well.

DNA-dependent transcriptional regulation in epithelium: new insights on the role of transactivation in the effect of glucocorticoids on immune responses

The advent of genome-scale hybridization studies has been critical to achieve a global view of the effect of glucocorticoids on gene expression. In different experimental settings, the use of microarray technology has clearly shown that glucocorticoid action displays a parallel inhibitory and enhancing effect on gene expression, with the activation effect equally represented, or even quantitatively larger than the inhibitory effect.[81,82] Most importantly, in these studies, glucocorticoid-regulated gene induction—validated by PCR and functional assays—is elicited at glucocorticoid concentrations considered suboptimal or low (10^{-7} to 10^{-8} mol/L) and believed so far to be associated almost exclusively with transrepression. Comprehensive reviews on glucocorticoid-induced genes have been published recently.[118,154] Recent studies have identified the induction, in airway epithelial cells, of 2 known glucocorticoid-inducible genes, glucocorticoid-induced leucine zipper (GILZ) and MKP-1, and have also identified the RNA-binding protein tristetraprolin as a glucocorticoid-induced gene. We discuss their diverse functions and potential relevance in the epithelial response to glucocorticoids, because they represent well the multiplicity of mechanisms implemented by glucocorticoids to carry out their anti-inflammatory action (Fig 2).

Glucocorticoid-induced leucine zipper is expressed on glucocorticoid treatment in T cells, mast cells, eosinophils, monocytes, and epithelial cells.[155,156] Overexpression of GILZ in T cells, monocytes, and DCs *in vitro*, as well as in T-cell–transgenic mice, mimics some of the anti-inflammatory and immunomodulatory effects that glucocorticoid exerts on these cells (see review[90,118]). The induction of GILZ by glucocorticoids occurs through binding of dimerized GR to its promoter region, which contains several GREs (see review[90]). GILZ mediates transcriptional control by glucocorticoids by interaction with Fos, Jun, and p65. Association of GILZ inhibits both DNA binding and transcriptional activation by AP-1 and NF-κB *in vitro* and in GILZ-transgenic mice,[157,158] thus providing glucocorticoids with potential transcriptional control on a large number of immune mediators. Furthermore, GILZ inhibits phosphorylation of the protein kinase Raf-1, therefore blocking the downstream activation of the MAP kinases, extracellular signal-regulated kinase (Erk) 1 and Erk 2.[159] In airway epithelial primary cells and cell lines (A549 and BEAS-2B), GILZ expression was upregulated by glucocorticoids and partially inhibited by IFN-γ, IL-1β, and TNF-α alone or in combination. Overexpression of GILZ inhibited cytokine-induced activation of a luciferase NF-κB reporter construct. Silencing of glucocorticoid-induced GILZ expression abrogated the inhibitory effect of glucocorticoid on IL-1β–induced IL-8/CXCL8 mRNA expression in BEAS-2B cells.[155] Therefore, GILZ has a potentially relevant anti-inflammatory role as a negative regulator of NF-κB activity in airway epithelium. However, GILZ also increases the expression and activity of the epithelial sodium channel α,[160] which is thought to contribute to glucocorticoid-mediated hypertension. Future availability of a $GILZ^{-/-}$ mouse model could help clarify the net contribution of GILZ to glucocorticoid action and to the control of NF-κB–mediated epithelial responses.

Carrying the glucocorticoid effect beyond the nucleus: glucocorticoid induction of MKP-1 and tristetraprolin

The action of glucocorticoids over immune responses is achieved in part through inhibition of the activity of members of the MAPK family, in particular of the stress-activated protein kinase (SAPK)/JNK, and p38/SAPK2. These kinases regulate gene expression through transcriptional as well as posttranscriptional mechanisms, by phosphorylation of transcription factors and RNA-binding proteins.[161] The inhibitory effect of glucocorticoids on these pathways is mediated at least in part by the induction, in many cell types including airway epithelium, of the MAPK phosphatase MKP-1 (also named dual-specificity phosphatase 1). By limiting MAPK signaling through dephosphorylation of MAPK members, glucocorticoid-induced MKP-1 limits the expression of inflammatory genes. Studies with transgenic and knockout models of MKP-1 have confirmed that this molecule is an important negative regulator of inflammation (see review[162,163]). Induction of MKP-1 by glucocorticoids is potentially a relevant anti-inflammatory mechanism, as

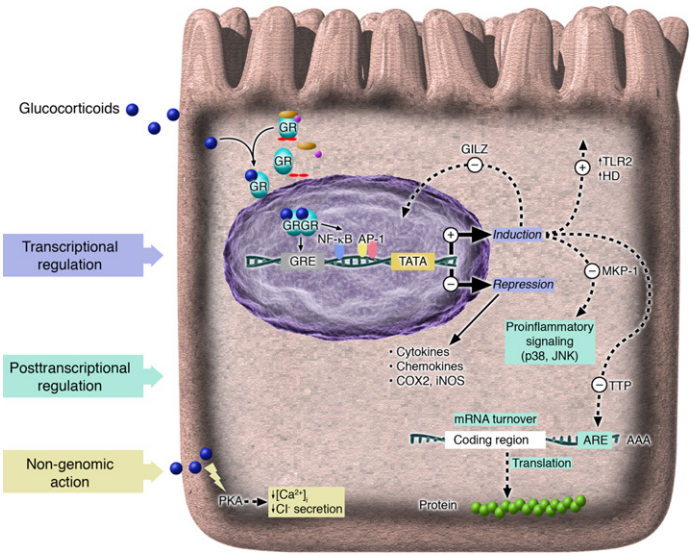

FIG 2. Mechanisms of glucocorticoid action in epithelial cells as discussed in this review. Glucocorticoids exert their anti-inflammatory action through multiple mechanisms mediating coordinate changes in gene expression. Binding of glucocorticoids activates the GR, which disassociates from a multimeric complex with chaperone proteins and translocates in the nucleus. Ligand-activated, dimerized GR acts as a transcription factor, controlling gene expression at transcriptional level *(purple boxes)* by either DNA-dependent mechanisms, mediated by binding to GRE—or in rare instances to nGRE—or by DNA-independent mechanisms. In the latter case, GR engages in protein:protein interactions with subunits of NF-κB, AP-1, and other transcription factors and cofactors, producing either transcriptional repression (−) of inflammatory genes, or induction (+) of genes involved in host defense such as TLR-2. Glucocorticoid-induced genes can also carry an inhibitory function by acting as transcriptional inhibitors (GILZ) or by mediating inhibitory glucocorticoid effects downstream of transcription. Among these, induction of MKP-1 mediates inhibition of cytokine signaling, mediated by members of the Erk/SAPK family; induction of tristetraprolin and MKP-1 mediates posttranscriptional effects of glucocorticoids *(green boxes)* on mRNA turnover. Finally, some antisecretory effects of glucocorticoids in epithelium appear to be mediated by rapid, nongenomic mechanisms *(yellow boxes)* independently from modulation of gene expression. *PKA*, Protein kinase A.

suggested by the significant impairment of glucocorticoid response in macrophages of MKP-$1^{-/-}$ mice.[131] As mentioned, the induction of MKP-1 mediates the glucocorticoid-induced increase in epithelial TLR-2.[84,85] Studies by Lasa et al[164] indicated that glucocorticoid-induced MKP-1 could extend glucocorticoid control to posttranscriptional gene regulatory mechanisms. In fact, MKP-1 was found to inhibit the p38-mediated process of stabilization of COX-2 mRNA. The mechanism of the induction of MKP-1 by glucocorticoids is yet unclear, because its promoter region is devoid of canonical GREs.[90] Given the large effect of MAPK signaling in epithelial response in inflammation, further studies on this glucocorticoid-induced pathway will likely yield important mechanistic insights on how epithelial gene expression is controlled by glucocorticoids.

Transcriptional control by glucocorticoids is integrated with action on posttranscriptional gene regulation, which modulates the rates of mRNA transport, decay, and translation and is critical in determining the timing and magnitude of the inflammatory response.[122] Inflammatory stimuli lead to phosphorylation-dependent transcript stabilization of cytokines and other inflammatory mediators, leading to a rapid rise of steady-state levels and protein translation; in balance with this action, pathways promoting mRNA decay are in place to limit the amplitude and the duration of the inflammatory response.[161] Genome-wide studies indicate that as many as 50% of genes induced during a stress response are mainly regulated through posttranscriptional means.[165] Studies in the airway epithelial cell line BEAS-2B indicate that a similar percentage of genes appear to be regulated by glucocorticoids through a posttranscriptional component.[166] The RNA binding protein tristetraprolin (also known as TIS11, Nup475, and GOS24), is a member of a family of CCCH zinc finger proteins that include tristetraprolin, butyrate-response factor (BRF)–1, and BRF-2.[167] Tristetraprolin is an early response gene induced in many cell types by inflammatory mediators[168] and promotes the mRNA decay of inflammatory mediators such as TNF-α,[169] GM-CSF, COX-2, iNOS, and more[170,171] through binding to an adenylate-urydilate-rich element (ARE) motif containing the UAUUUAU heptamer, which is present in the 3′ untranslated region (UTR) of the mRNA.[172] Mice deficient in tristetraprolin display early onset of cachexia, severe inflammatory arthritis, autoimmune dysfunction, and myeloid hyperplasia through the deregulated expression of TNF-α and GM-CSF,[173] clearly indicating the importance of tristetraprolin in limiting the inflammatory response.

In a study screening the effect of glucocorticoids on the expression of RNA-binding proteins, tristetraprolin and BRF-1 were found to be induced by glucocorticoids in

primary airway epithelial cells and in the bronchial epithelial cell line BEAS-2B.[133] Pawliczak et al[174] reported tristetraprolin among the transcripts induced by glucocorticoids in normal human bronchial epithelial cells in a genome-wide analysis of glucocorticoid-mediated gene expression. Furthermore, Smoak and Cidlowski[132] have recently demonstrated that glucocorticoids induce the production of tristetraprolin *in vivo* in several organs in mice, including the lungs, as well as in the airway A549 human epithelial cell line. Nuclear run-on experiments in A549 cells indicated that dexamethasone induced transcription of tristetraprolin, and chromatin immunoprecipitation assay identified association of the GR with areas of the 5′ DNA flanking region of the tristetraprolin gene containing consensus sites for STAT, SMA mothers against decapentaplegic, and NF-κB, and with an area on the 3′ end containing a GRE half-site. Silencing of tristetraprolin expression by siRNA in A549 cells significantly impaired glucocorticoid inhibition of TNF-α expression; this process was dependent on the ARE-bearing TNF-α 3′ UTR, because treatment with dexamethasone selectively decreased the expression of a luciferase reporter carrying the TNF-α 3′ UTR. Together with studies on the posttranscriptional effect of glucocorticoids on COX-2 expression,[164] these data point at ARE-mediated control of transcript stability as an important yet understudied aspect of glucocorticoid action in epithelium. Along these lines, a genome-wide study indicated that glucocorticoid response in tristetraprolin$^{-/-}$ mouse embryonic fibroblasts is profoundly impaired, because about 85% of the overall changes after glucocorticoid treatment were lost in comparison with the robust response elicited in wild-type mouse embryonic fibroblasts.[133]

As in the case of GILZ, tristetraprolin is also induced both by glucocorticoid and inflammatory stimuli, the latter presumably serving as an endogenous mediator that limits the inflammatory response. To establish firmly the relevance of glucocorticoid-induced proteins such as GILZ, MKP-1, and tristetraprolin as mediators of glucocorticoid action in epithelium, it will be important to implement clinical studies to examine the levels of these proteins in the airways of normal and well characterized patients with asthma and their modulation after glucocorticoid therapy, and to test their role in different murine models of inflammation.

An epithelial gene controlled by glucocorticoids through a negative GRE

Within DNA-dependent control of gene expression, the occurrence of an inhibitory effect of glucocorticoid mediated by a GRE, indicated as a negative (n) GRE, is rare. Edwards et al[175] report that in airway epithelial cells, induction of IL-6 by rhinovirus is dependent on a single nGRE site proximal to the TATA box and to the transcription start site. Mutation of this consensus site led to a loss of inhibition by fluticasone of a functional IL-6 promoter construct. The authors of this study speculate that binding of the dimerized GR to the nGRE element could block the function of the RNA polymerase at the transcription start site, either by steric hindrance or by preventing the binding of other transcription factors and the subsequent formation of the complex with coactivators, such as CBP or p300, which are necessary to implement transcription.

SUMMARY

Glucocorticoids exert a profound effect on the immune functions performed by epithelial cells in innate and adaptive immunity. It is now well established by studies *in vitro* and *in vivo* that glucocorticoids provide a substantial inhibition of the proinflammatory response mounted by the epithelium in response to triggers produced by inflammatory cells, and that the control of this epithelial-driven response is an important component of the efficacy of glucocorticoid therapy in asthma and other allergic diseases of the airways. More recent studies have characterized the equally relevant protective role that glucocorticoids play in the innate immune response of the epithelium, which is a function of key importance in the biology of this cell type, given its direct contact with the external world. Primary alterations of the epithelial layer, caused by genetic susceptibility and/or by early exposure to viruses, as well as alteration of signaling brought by inflammation may converge to create a distinct cell phenotype displaying a lack of response to glucocorticoids in epithelial cells of patients with severe asthma. As genomic and biochemical studies keep extending the complexity of the effect of glucocorticoid on gene expression, it is clear that the mechanisms mediating GR effects involve multiple complex regulatory interactions, such as those between glucocorticoid-induced MKP-1 and negative regulatory MAPKs, that are yet to be fully uncovered. The contribution of the different mechanisms of gene regulation implemented by glucocorticoids, discussed in this review, may integrate in a dynamic fashion, according to the type of ongoing response, the cellular target, and the genes involved.

To exploit the epithelial response to glucocorticoids for therapeutics, we will need a better understanding of these complex interactions among regulatory proteins—transcription factors, coactivators and corepressors, and RNA-binding proteins—occurring during the allergic response at the transcriptional and posttranscriptional levels of gene regulation; similarly, a better understanding of the role of GRβ and additional knowledge on the identity of the epithelial isoforms of GRα will help define the specific range of response that glucocorticoids exert in epithelial cells, as well as their contribution to the multicellular phenotypic changes underlying resistance to glucocorticoid therapy.

I sincerely thank Dr John A. Cidlowski for critically reviewing the manuscript. I am indebted to all authors' work, from which I learned a great deal in preparing this review, and I apologize for not including all of the relevant publications in the reference list (because of space limitations).

REFERENCES

1. Knight DA, Holgate ST. The airway epithelium: structural and functional properties in health and disease. Respirology 2003;8:432-46.
2. Holtzman MJ, Morton JD, Shornick LP, Tyner JW, O'Sullivan MP, Antao A, et al. Immunity, inflammation, and remodeling in the airway epithelial barrier: epithelial-viral-allergic paradigm. Physiol Rev 2002;82:19-46.
3. Chanez P. Severe asthma is an epithelial disease. Eur Respir J 2005;25: 945-6.
4. Holgate ST. The epithelium takes centre stage in asthma and atopic dermatitis. Trends Immunol 2007;28:248-51.
5. Cookson W. The immunogenetics of asthma and eczema: a new focus on the epithelium. Nat Rev Immunol 2004;4:978-88.
6. Schleimer RP, O'Byrne PM, Szefler SJ, Brattsand R, editors. Inhaled steroids in asthma: optimizing effects in the airways. New York, Basel: Marcel Dekker; 2002.
7. Stellato C, Schwiebert LM, Schleimer RP. Mechanisms of glucocorticosteroid action. In: Barnes PJ, Grunstein MM, Leff A, Woolcock AJ, editors. Asthma. Philadelphia: Lippincott-Raven; 1997. p. 1569-96.
8. Schleimer RP. Glucocorticoids suppress inflammation but spare innate immune responses in airway epithelium. Proc Am Thorac Soc 2004;1: 222-30.
9. Zhang N, Truong-Tran QA, Tancowny B, Harris KE, Schleimer RP. Glucocorticoids enhance or spare innate immunity: effects in airway epithelium are mediated by CCAAT/enhancer binding proteins. J Immunol 2007;179:578-89.
10. Evans MJ, Van Winkle LS, Fanucchi MV, Plopper CG. Cellular and molecular characteristics of basal cells in airway epithelium. Exp Lung Res 2001;27:401-15.
11. Puddicombe SM, Torres-Lozano C, Richter A, Bucchieri F, Lordan JL, Howarth PH, et al. Increased expression of p21(waf) cyclin-dependent kinase inhibitor in asthmatic bronchial epithelium. Am J Respir Cell Mol Biol 2003;28:61-8.
12. Mellow TE, Murphy PC, Carson JL, Noah TL, Zhang L, Pickles RJ. The effect of respiratory synctial virus on chemokine release by differentiated airway epithelium. Exp Lung Res 2004;30:43-57.
13. Del Donno M, Bittesnich D, Chetta A, Olivieri D, Lopez-Vidriero MT. The effect of inflammation on mucociliary clearance in asthma: an overview. Chest 2000;118:1142-9.
14. Fahy JV. Goblet cell and mucin gene abnormalities in asthma. Chest 2002;122:320S-6S.
15. Hong KU, Reynolds SD, Giangreco A, Hurley CM, Stripp BR. Clara cell secretory protein-expressing cells of the airway neuroepithelial body microenvironment include a label-retaining subset and are critical for epithelial renewal after progenitor cell depletion. Am J Respir Cell Mol Biol 2001;24:671-81.
16. Kouznetsova I, Chwieralski CE, Balder R, Hinz M, Braun A, Krug N, et al. Induced trefoil factor family 1 expression by trans-differentiating Clara cells in a murine asthma model. Am J Respir Cell Mol Biol 2007; 36:286-95.
17. Homer RJ, Zhu Z, Cohn L, Lee CG, White WI, Chen S, et al. Differential expression of chitinases identify subsets of murine airway epithelial cells in allergic inflammation. Am J Physiol Lung Cell Mol Physiol 2006;291:L502-11.
18. Zhao J, Yeong LH, Wong WS. Dexamethasone alters bronchoalveolar lavage fluid proteome in a mouse asthma model. Int Arch Allergy Immunol 2007;142:219-29.
19. Dulkerian SJ, Gonzales LW, Ning Y, Ballard PL. Regulation of surfactant protein D in human fetal lung. Am J Respir Cell Mol Biol 1996;15:781-6.
20. Sano H, Kuroki Y. The lung collectins, SP-A and SP-D, modulate pulmonary innate immunity. Mol Immunol 2005;42:279-87.
21. Boers JE, den Brok JL, Koudstaal J, Arends JW, Thunnissen FB. Number and proliferation of neuroendocrine cells in normal human airway epithelium. Am J Respir Crit Care Med 1996;154:758-63.
22. Adriaensen D, Brouns I, Van Genechten J, Timmermans JP. Functional morphology of pulmonary neuroepithelial bodies: extremely complex airway receptors. Anat Rec A Discov Mol Cell Evol Biol 2003;270:25-40.
23. Sunday ME, Shan L, Subramaniam M. Immunomodulatory functions of the diffuse neuroendocrine system: implications for bronchopulmonary dysplasia. Endocr Pathol 2004;15:91-106.
24. Dayer AM, Kapanci Y, Rademakers A, Rusy LM, De Mey J, Will JA. Increased numbers of neuroepithelial bodies (NEB) in lungs of fetal rhesus monkeys following maternal dexamethasone treatment. Cell Tissue Res 1985;239:703-5.
25. Pu FR, Manning FC, Brannigan AE, Crosby SR. Differential regulation of calcitonin secretion in normal and neoplastic pulmonary neuroendocrine cells in vitro. Exp Lung Res 2001;27:689-703.
26. Laitinen A, Karjalainen EM, Altraja A, Laitinen LA. Histopathologic features of early and progressive asthma. J Allergy Clin Immunol 2000;105:S509-13.
27. Laitinen LA, Laitinen A, Haahtela T. Airway mucosal inflammation even in patients with newly diagnosed asthma. Am Rev Respir Dis 1993;147:697-704.
28. Shebani E, Shahana S, Janson C, Roomans GM. Attachment of columnar airway epithelial cells in asthma. Tissue Cell 2005;37:145-52.
29. Trautmann A, Kruger K, Akdis M, Muller-Wening D, Akkaya A, Brocker EB, et al. Apoptosis and loss of adhesion of bronchial epithelial cells in asthma. Int Arch Allergy Immunol 2005;138:142-50.
30. Montefort S, Djukanovic R, Holgate ST, Roche WR. Ciliated cell damage in the bronchial epithelium of asthmatics and non-asthmatics. Clin Exp Allergy 1993;23:185-9.
31. Bucchieri F, Puddicombe SM, Lordan JL, Richter A, Buchanan D, Wilson SJ, et al. Asthmatic bronchial epithelium is more susceptible to oxidant-induced apoptosis. Am J Respir Cell Mol Biol 2002;27:179-85.
32. Vignola AM, Chiappara G, Siena L, Bruno A, Gagliardo R, Merendino AM, et al. Proliferation and activation of bronchial epithelial cells in corticosteroid-dependent asthma. J Allergy Clin Immunol 2001;108:738-46.
33. Cohen L, E X, Tarsi J, Ramkumar T, Horiuchi TK, Cochran R, et al. Epithelial cell proliferation contributes to airway remodeling in severe asthma. Am J Respir Crit Care Med 2007;176:138-45.
34. Chetta A, Foresi A, Del Donno M, Bertorelli G, Pesci A, Olivieri D. Airways remodeling is a distinctive feature of asthma and is related to severity of disease. Chest 1997;111:852-7.
35. Vignola AM, Campbell AM, Chanez P, Bousquet J, Paul-Lacoste P, Michel FB, et al. HLA-DR and ICAM-1 expression on bronchial epithelial cells in asthma and chronic bronchitis. Am Rev Respir Dis 1993;148:689-94.
36. Vachier I, Chiappara G, Vignola AM, Gagliardo R, Altieri E, Terouanne B, et al. Glucocorticoid receptors in bronchial epithelial cells in asthma. Am J Respir Crit Care Med 1998;158:963-70.
37. Puddicombe SM, Polosa R, Richter A, Krishna MT, Howarth PH, Holgate ST, et al. Involvement of the epidermal growth factor receptor in epithelial repair in asthma. FASEB J 2000;14:1362-74.
38. Hamilton LM, Puddicombe SM, Dearman RJ, Kimber I, Sandstrom T, Wallin A, et al. Altered protein tyrosine phosphorylation in asthmatic bronchial epithelium. Eur Respir J 2005;25:978-85.
39. Laitinen LA, Laitinen A, Haahtela T. A comparative study of the effects of an inhaled corticosteroid, budesonide, and a beta 2-agonist, terbutaline, on airway inflammation in newly diagnosed asthma: a randomized, double-blind, parallel-group controlled trial. J Allergy Clin Immunol 1992;90:32-42.
40. Prosperini G, Rajakulasingam K, Cacciola RR, Spicuzza L, Rorke S, Holgate ST, et al. Changes in sputum counts and airway hyperresponsiveness after budesonide: monitoring anti-inflammatory response on the basis of surrogate markers of airway inflammation. J Allergy Clin Immunol 2002;110:855-61.
41. Heino M, Karjalainen J, Ylikoski J, Laitinen A, Laitinen LA. Bronchial ciliogenesis and oral steroid treatment in patients with asthma. Br J Dis Chest 1988;82:175-8.
42. Lundgren R, Soderberg M, Horstedt P, Stenling R. Morphological studies of bronchial mucosal biopsies from asthmatics before and after ten years of treatment with inhaled steroids. Eur Respir J 1988;1:883-9.
43. Stellato C, Schleimer RP. Regulation of chemokines by glucocorticoids. In: Rothenberg ME, editor. Chemokines in allergic disease. New York, Basel: Marcel Dekker; 2000. p. 473-507.
44. Frigas E, Loegering DA, Solley GO, Farrow GM, Gleich GJ. Elevated levels of the eosinophil granule major basic protein in the sputum of patients with bronchial asthma. Mayo Clin Proc 1981;56:345-53.
45. Carayol N, Campbell A, Vachier I, Mainprice B, Bousquet J, Godard P, et al. Modulation of cadherin and catenins expression by tumor necrosis factor-alpha and dexamethasone in human bronchial epithelial cells. Am J Respir Cell Mol Biol 2002;26:341-7.
46. Chakir J, Shannon J, Molet S, Fukakusa M, Elias J, Laviolette M, et al. Airway remodeling-associated mediators in moderate to severe asthma:

effect of steroids on TGF-beta, IL-11, IL-17, and type I and type III collagen expression. J Allergy Clin Immunol 2003;111:1293-8.
47. Aikawa T, Shimura S, Sasaki H, Ebina M, Takishima T. Marked goblet cell hyperplasia with mucus accumulation in the airways of patients who died of severe acute asthma attack. Chest 1992;101:916-21.
48. Groneberg DA, Eynott PR, Lim S, Oates T, Wu R, Carlstedt I, et al. Expression of respiratory mucins in fatal status asthmaticus and mild asthma. Histopathology 2002;40:367-73.
49. Voynow JA, Gendler SJ, Rose MC. Regulation of mucin genes in chronic inflammatory airway diseases. Am J Respir Cell Mol Biol 2006;34:661-5.
50. Rose MC, Voynow JA. Respiratory tract mucin genes and mucin glycoproteins in health and disease. Physiol Rev 2006;86:245-78.
51. Rogers DF. Physiology of airway mucus secretion and pathophysiology of hypersecretion. Respir Care 2007;52:1134-49.
52. Barnes PJ, Adcock I. Anti-inflammatory actions of steroids: molecular mechanisms. Trends Pharmacol Sci 1993;14:436-41.
53. Blyth DI, Pedrick MS, Savage TJ, Bright H, Beesley JE, Sanjar S. Induction, duration, and resolution of airway goblet cell hyperplasia in a murine model of atopic asthma: effect of concurrent infection with respiratory syncytial virus and response to dexamethasone. Am J Respir Cell Mol Biol 1998;19:38-54.
54. Blyth DI, Pedrick MS, Savage TJ, Hessel EM, Fattah D. Lung inflammation and epithelial changes in a murine model of atopic asthma. Am J Respir Cell Mol Biol 1996;14:425-38.
55. Kibe A, Inoue H, Fukuyama S, Machida K, Matsumoto K, Koto H, et al. Differential regulation by glucocorticoid of interleukin-13-induced eosinophilia, hyperresponsiveness, and goblet cell hyperplasia in mouse airways. Am J Respir Crit Care Med 2003;167:50-6.
56. Lu W, Lillehoj EP, Kim KC. Effects of dexamethasone on Muc5ac mucin production by primary airway goblet cells. Am J Physiol Lung Cell Mol Physiol 2005;288:L52-60.
57. Chen Y, Nickola TJ, DiFronzo NL, Colberg-Poley AM, Rose MC. Dexamethasone-mediated repression of MUC5AC gene expression in human lung epithelial cells. Am J Respir Cell Mol Biol 2006;34:338-47.
58. Kuperman DA, Huang X, Koth LL, Chang GH, Dolganov GM, Zhu Z, et al. Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. Nat Med 2002;8:885-9.
59. Heller NM, Matsukura S, Georas SN, Boothby MR, Stellato C, Schleimer RP. Assessment of signal transducer and activator of transcription 6 as a target of glucocorticoid action in human airway epithelial cells. Clin Exp Allergy 2004;34:1690-700.
60. Zhou Y, Dong Q, Louahed J, Dragwa C, Savio D, Huang M, et al. Characterization of a calcium-activated chloride channel as a shared target of Th2 cytokine pathways and its potential involvement in asthma. Am J Respir Cell Mol Biol 2001;25:486-91.
61. Dolganov GM, Woodruff PG, Novikov AA, Zhang Y, Ferrando RE, Szubin R, et al. A novel method of gene transcript profiling in airway biopsy homogenates reveals increased expression of a Na+-K+-Cl- cotransporter (NKCC1) in asthmatic subjects. Genome Res 2001;11:1473-83.
62. Gibson A, Lewis AP, Affleck K, Aitken AJ, Meldrum E, Thompson N. hCLCA1 and mCLCA3 are secreted non-integral membrane proteins and therefore are not ion channels. J Biol Chem 2005;280:27205-12.
63. Urbach V, Verriere V, Grumbach Y, Bousquet J, Harvey BJ. Rapid anti-secretory effects of glucocorticoids in human airway epithelium. Steroids 2006;71:323-8.
64. O'Riordan TG, Mao Y, Otero R, Lopez J, Sabater JR, Abraham WM. Budesonide affects allergic mucociliary dysfunction. J Appl Physiol 1998;85:1086-91.
65. Shah RV, Amin M, Sangwan S, Smaldone GC. Steroid effects on mucociliary clearance in outpatient asthma. J Aerosol Med 2006;19:208-20.
66. Agnew JE, Bateman JR, Pavia D, Clarke SW. Peripheral airways mucus clearance in stable asthma is improved by oral corticosteroid therapy. Bull Eur Physiopathol Respir 1984;20:295-301.
67. Takeyama K, Dabbagh K, Lee HM, Agusti C, Lausier JA, Ueki IF, et al. Epidermal growth factor system regulates mucin production in airways. Proc Natl Acad Sci U S A 1999;96:3081-6.
68. Nadel JA. Innate immune mucin production via epithelial cell surface signaling: relationship to allergic disease. Curr Opin Allergy Clin Immunol 2007;7:57-62.
69. Park KS, Korfhagen TR, Bruno MD, Kitzmiller JA, Wan H, Wert SE, et al. SPDEF regulates goblet cell hyperplasia in the airway epithelium. J Clin Invest 2007;117:978-88.
70. Sansonetti PJ. The innate signaling of dangers and the dangers of innate signaling. Nat Immunol 2006;7:1237-42.
71. Hippenstiel S, Opitz B, Schmeck B, Suttorp N. Lung epithelium as a sentinel and effector system in pneumonia: molecular mechanisms of pathogen recognition and signal transduction. Respir Res 2006;7:97-114.
72. Bals R, Hiemstra PS. Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. Eur Respir J 2004;23:327-33.
73. Wark PA, Johnston SL, Bucchieri F, Powell R, Puddicombe S, Laza-Stanca V, et al. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. J Exp Med 2005;201:937-47.
74. Contoli M, Message SD, Laza-Stanca V, Edwards MR, Wark PA, Bartlett NW, et al. Role of deficient type III interferon-lambda production in asthma exacerbations. Nat Med 2006;12:1023-6.
75. Gonzalez Rodriguez R, Silvestri M, Cordone A, Salami A, Rossi GA. Inhibition of eosinophil transepithelial migration and downregulation of adhesion molecule expression on eosinophils and airway epithelial cells induced by budesonide. Pulm Pharmacol Ther 2000;13:31-8.
76. Shirasaki H, Watanabe K, Kanaizumi E, Sato J, Konno N, Narita S, et al. Effect of glucocorticosteroids on tumour necrosis factor-alpha-induced intercellular adhesion molecule-1 expression in cultured primary human nasal epithelial cells. Clin Exp Allergy 2004;34:945-51.
77. Grunberg K, Sharon RF, Hiltermann TJ, Brahim JJ, Dick EC, Sterk PJ, et al. Experimental rhinovirus 16 infection increases intercellular adhesion molecule-1 expression in bronchial epithelium of asthmatics regardless of inhaled steroid treatment. Clin Exp Allergy 2000;30:1015-23.
78. Traves SL, Proud D. Viral-associated exacerbations of asthma and COPD. Curr Opin Pharmacol 2007;7:252-8.
79. Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. N Engl J Med 1995;332:1351-62.
80. Calverley PM. Effect of corticosteroids on exacerbations of asthma and chronic obstructive pulmonary disease. Proc Am Thorac Soc 2004;1:161-6.
81. Galon J, Franchimont D, Hiroi N, Frey G, Boettner A, Ehrhart-Bornstein M, et al. Gene profiling reveals unknown enhancing and suppressive actions of glucocorticoids on immune cells. FASEB J 2002;16:61-71.
82. Ehrchen J, Steinmuller L, Barczyk K, Tenbrock K, Nacken W, Eisenacher M, et al. Glucocorticoids induce differentiation of a specifically activated, anti-inflammatory subtype of human monocytes. Blood 2007;109:1265-74.
83. Perretti M. Glucocorticoids in innate immunity: more transactivation than transrepression! Blood 2007;109:852-3.
84. Imasato A, Desbois-Mouthon C, Han J, Kai H, Cato AC, Akira S, et al. Inhibition of p38 MAPK by glucocorticoids via induction of MAPK phosphatase-1 enhances nontypeable Haemophilus influenzae-induced expression of toll-like receptor 2. J Biol Chem 2002;277:47444-50.
85. Shuto T, Imasato A, Jono H, Sakai A, Xu H, Watanabe T, et al. Glucocorticoids synergistically enhance nontypeable Haemophilus influenzae-induced Toll-like receptor 2 expression via a negative cross-talk with p38 MAP kinase. J Biol Chem 2002;277:17263-70.
86. Hermoso MA, Matsuguchi T, Smoak K, Cidlowski JA. Glucocorticoids and tumor necrosis factor alpha cooperatively regulate toll-like receptor 2 gene expression. Mol Cell Biol 2004;24:4743-56.
87. Homma T, Kato A, Hashimoto N, Batchelor J, Yoshikawa M, Imai S, et al. Corticosteroid and cytokines synergistically enhance toll-like receptor 2 expression in respiratory epithelial cells. Am J Respir Cell Mol Biol 2004;31:463-9.
88. Musikacharoen T, Matsuguchi T, Kikuchi T, Yoshikai Y. NF-kappa B and STAT5 play important roles in the regulation of mouse Toll-like receptor 2 gene expression. J Immunol 2001;166:4516-24.
89. Bornstein SR, Ziegler CG, Krug AW, Kanczkowski W, Rettori V, McCann SM, et al. The role of Toll-like receptors in the immune-adrenal crosstalk. Ann N Y Acad Sci 2006;1088:307-18.
90. Chinenov Y, Rogatsky I. Glucocorticoids and the innate immune system: crosstalk with the Toll-like receptor signaling network. Mol Cell Endocrinol 2007;275:30-42.
91. Lambrecht BN, Hammad H. The other cells in asthma: dendritic cell and epithelial cell crosstalk. Curr Opin Pulm Med 2003;9:34-41.

92. Pichavant M, Charbonnier AS, Taront S, Brichet A, Wallaert B, Pestel J, et al. Asthmatic bronchial epithelium activated by the proteolytic allergen Der p 1 increases selective dendritic cell recruitment. J Allergy Clin Immunol 2005;115:771-8.
93. Pichavant M, Taront S, Jeannin P, Breuilh L, Charbonnier AS, Spriet C, et al. Impact of bronchial epithelium on dendritic cell migration and function: modulation by the bacterial motif KpOmpA. J Immunol 2006;177:5912-9.
94. Allakhverdi Z, Comeau MR, Jessup HK, Yoon BR, Brewer A, Chartier S, et al. Thymic stromal lymphopoietin is released by human epithelial cells in response to microbes, trauma, or inflammation and potently activates mast cells. J Exp Med 2007;204:253-8.
95. Lee HC, Ziegler SF. Inducible expression of the proallergic cytokine thymic stromal lymphopoietin in airway epithelial cells is controlled by NFkappaB. Proc Natl Acad Sci U S A 2007;104:914-9.
96. Kato A, Favoreto S Jr, Avila PC, Schleimer RP. TLR3- and Th2 cytokine-dependent production of thymic stromal lymphopoietin in human airway epithelial cells. J Immunol 2007;179:1080-7.
97. Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. Nat Immunol 2002;3:673-80.
98. Ying S, O'Connor B, Ratoff J, Meng Q, Mallett K, Cousins D, et al. Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. J Immunol 2005;174:8183-90.
99. Proud D, Sanders SP, Wiehler S. Human rhinovirus infection induces airway epithelial cell production of human [beta]-defensin 2 both in vitro and in vivo. J Immunol 2004;172:4637-45.
100. Roca-Ferrer J, Mullol J, Lopez E, Xaubet A, Pujols L, Fernandez JC, et al. Effect of topical anti-inflammatory drugs on epithelial cell-induced eosinophil survival and GM-CSF secretion. Eur Respir J 1997;10: 1489-95.
101. Adkins KK, Levan TD, Miesfeld RL, Bloom JW. Glucocorticoid regulation of GM-CSF: evidence for transcriptional mechanisms in airway epithelial cells. Am J Physiol 1998;275:L372-8.
102. Schleimer RP. Glucocorticosteroids: their mechanisms of action and use in allergic diseases. In: Middleton E, Ellis EF, Adkinson NF, Yunginger JW, Busse W, editors. Allergy: principles and practice. St Louis (MO): Mosby; 1998. p. 638-60.
103. Sekiya T, Miyamasu M, Imanishi M, Yamada H, Nakajima T, Yamaguchi M, et al. Inducible expression of a Th2-type CC chemokine thymus- and activation-regulated chemokine by human bronchial epithelial cells. J Immunol 2000;165:2205-13.
104. Stellato C, Matsukura S, Fal A, White J, Beck LA, Proud D, et al. Differential regulation of epithelial-derived C-C chemokine expression by IL-4 and the glucocorticoid budesonide. J Immunol 1999;163: 5624-32.
105. Spurrell JCL, Wiehler S, Zaheer RS, Sanders SP, Proud D. Human airway epithelial cells produce IP-10 (CXCL10) in vitro and in vivo upon rhinovirus infection. Am J Physiol Lung Cell Mol Physiol 2005;289: L85-95.
106. Sauty A, Dziejman M, Taha RA, Iarossi AS, Neote K, Garcia-Zepeda EA, et al. The T cell-specific CXC chemokines IP-10, Mig, and I-TAC are expressed by activated human bronchial epithelial cells. J Immunol 1999;162:3549-58.
107. Wang JH, Devalia JL, Xia C, Sapsford RJ, Davies RJ. Expression of RANTES by human bronchial epithelial cells in vitro and in vivo and the effect of corticosteroids. Am J Respir Cell Mol Biol 1996;14: 27-35.
108. Stellato C, Beck LA, Gorgone GA, Proud D, Schall TJ, Ono SJ, et al. Expression of the chemokine RANTES by a human bronchial epithelial cell line: modulation by cytokines and glucocorticoids. J Immunol 1995;155:410-8.
109. Edwards MR, Johnson MW, Johnston SL. Combination therapy: synergistic suppression of virus-induced chemokines in airway epithelial cells. Am J Respir Cell Mol Biol 2006;34:616-24.
110. Wilson SJ, Wallin A, Della-Cioppa G, Sandstrom T, Holgate ST. Effects of budesonide and formoterol on NF-kappaB, adhesion molecules, and cytokines in asthma. Am J Respir Crit Care Med 2001;164: 1047-52.
111. Fukakusa M, Bergeron C, Tulic MK, Fiset P-O, Al Dewachi O, Laviolette M, et al. Oral corticosteroids decrease eosinophil and CC chemokine expression but increase neutrophil, IL-8, and IFN-[gamma]-inducible protein 10 expression in asthmatic airway mucosa. J Allergy Clin Immunol 2005;115:280-6.
112. Fan J, Heller NM, Gorospe M, Atasoy U, Stellato C. The role of post-transcriptional regulation in chemokine gene expression in inflammation and allergy. Eur Respir J 2005;26:933-47.
113. Holtzman MJ. Arachidonic acid metabolism in airway epithelial cells. Annu Rev Physiol 1992;54:303-29.
114. Newton R, Seybold J, Kuitert LM, Bergmann M, Barnes PJ. Repression of cyclooxygenase-2 and prostaglandin E2 release by dexamethasone occurs by transcriptional and post-transcriptional mechanisms involving loss of polyadenylated mRNA. J Biol Chem 1998;273: 32312-21.
115. Redington AE, Meng QH, Springall DR, Evans TJ, Creminon C, Maclouf J, et al. Increased expression of inducible nitric oxide synthase and cyclo-oxygenase-2 in the airway epithelium of asthmatic subjects and regulation by corticosteroid treatment. Thorax 2001; 56:351-7.
116. Aksoy MO, Li X, Borenstein M, Yi Y, Kelsen SG. Effects of topical corticosteroids on inflammatory mediator-induced eicosanoid release by human airway epithelial cells. J Allergy Clin Immunol 1999;103: 1081-91.
117. Kobzik L, Bredt DS, Lowenstein CJ, Drazen J, Gaston B, Sugarbaker D, et al. Nitric oxide synthase in human and rat lung: immunocytochemical and histochemical localization. Am J Respir Cell Mol Biol 1993;9: 371-7.
118. Clark AR. Anti-inflammatory functions of glucocorticoid-induced genes. Mol Cell Endocrinol 2007;275:79-97.
119. Ito K, Chung F, Adcock IH. Update on glucocorticoid action and resistance. J Allergy Clin Immunol 2006;117:522-43.
120. Barnes PJ. How corticosteroids control inflammation: Quintiles Prize Lecture 2005. Br J Pharmacol 2006;148:245-54.
121. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids: new mechanisms for old drugs. N Engl J Med 2005;353: 1711-23.
122. Stellato C. Post-transcriptional and nongenomic effects of glucocorticoids. Proc Am Thorac Soc 2004;1:255-63.
123. Lewis-Tuffin LJ, Cidlowski JA. The physiology of human glucocorticoid receptor beta (hGRbeta) and glucocorticoid resistance. Ann N Y Acad Sci 2006;1069:1-9.
124. Lewis-Tuffin LJ, Jewell CM, Bienstock RJ, Collins JB, Cidlowski JA. Human glucocorticoid receptor beta binds RU-486 and is transcriptionally active. Mol Cell Biol 2007;27:2266-82.
125. Lefstin JA, Yamamoto KR. Allosteric effects of DNA on transcriptional regulators. Nature 1998;392:885-8.
126. Li Q, Verma IM. NF-[kappa]B regulation in the immune system. Nat Rev Immunol 2002;2:725-34.
127. McKay LI, Cidlowski JA. Molecular control of immune/inflammatory responses: interactions between nuclear factor-{kappa}B and steroid receptor-signaling pathways. Endocr Rev 1999;20:435-59.
128. Karin M, Saatciouglu F. Negative transcriptional regulation by the glucocorticoid receptor is responsible for the antiinflammatory activity of glucocorticoids. In: Schleimer RP, Busse WW, O'Byrne PM, editors. Inhaled glucocorticoids in asthma: mechanisms and clinical actions. New York: Marcel Dekker, Inc; 1997. p. 29-52.
129. Rogatsky I, Ivashkiv LB. Glucocorticoid modulation of cytokine signaling. Tissue Ant 2006;68:1-12.
130. Adcock IM, Ito K, Barnes PJ. Glucocorticoids: effects on gene transcription. Proc Am Thorac Soc 2004;1:247-54.
131. Abraham SM, Lawrence T, Kleiman A, Warden P, Medghalchi M, Tuckermann J, et al. Antiinflammatory effects of dexamethasone are partly dependent on induction of dual specificity phosphatase 1. J Exp Med 2006;203:1883-9.
132. Smoak K, Cidlowski JA. Glucocorticoids regulate tristetraprolin synthesis and posttranscriptionally regulate tumor necrosis factor alpha inflammatory signaling. Mol Cell Biol 2006;26:9126-35.
133. Ishmael FT, Fang X, Heller NH, Fan J, Atasoy U, Cheadle C, et al. Role of the RNA-binding protein tristetraprolin (TTP) in glucocorticoid (GC)-mediated gene regulation [abstract]. J Allergy Clin Immunol 2007;119:S134.
134. Losel R, Wehling M. Nongenomic actions of steroid hormones. Nat Rev Mol Cell Biol 2003;4:46-56.

135. Wanner A, Horvath G, Brieva JL, Kumar SD, Mendes ES. Nongenomic actions of glucocorticosteroids on the airway vasculature in asthma. Proc Am Thorac Soc 2004;1:235-8.
136. LeVan TD, Babin EA, Yamamura HI, Bloom JW. Pharmacological characterization of glucocorticoid receptors in primary human bronchial epithelial cells. Biochem Pharmacol 1999;57:1003-9.
137. Adcock IM, Gilbey T, Gelder CM, Chung KF, Barnes PJ. Glucocorticoid receptor localization in normal and asthmatic lung. Am J Respir Crit Care Med 1996;154:771-82.
138. Sousa AR, Lane SJ, Cidlowski JA, Staynov DZ, Lee TH. Glucocorticoid resistance in asthma is associated with elevated in vivo expression of the glucocorticoid receptor beta-isoform. J Allergy Clin Immunol 2000;105:943-50.
139. Webster JC, Oakley RH, Jewell CM, Cidlowski JA. Proinflammatory cytokines regulate human glucocorticoid receptor gene expression and lead to the accumulation of the dominant negative beta isoform: a mechanism for the generation of glucocorticoid resistance. Proc Natl Acad Sci U S A 2001;98:6865-70.
140. Gagliardo R, Chanez P, Vignola AM, Bousquet J, Vachier I, Godard P, et al. Glucocorticoid receptor alpha and beta in glucocorticoid dependent asthma. Am J Respir Crit Care Med 2000;162:7-13.
141. Mathieu M. Is there a role for glucocorticoid receptor beta in glucocorticoid-dependent asthmatics? Am J Respir Crit Care Med 2001;163: 585-6.
142. Leung DY, Chrousos GP. Is there a role for glucocorticoid receptor beta in glucocorticoid-dependent asthmatics? Am J Respir Crit Care Med 2000;162:1-3.
143. Christodoulopoulos P, Leung DY, Elliott MW, Hogg JC, Muro S, Toda M, et al. Increased number of glucocorticoid receptor-beta-expressing cells in the airways in fatal asthma. J Allergy Clin Immunol 2000; 106:479-84.
144. Hamilos DL, Leung DY, Muro S, Kahn AM, Hamilos SS, Thawley SE, et al. GRbeta expression in nasal polyp inflammatory cells and its relationship to the anti-inflammatory effects of intranasal fluticasone. J Allergy Clin Immunol 2001;108:59-68.
145. Bergeron C, Fukakusa M, Olivenstein R, Lemiere C, Shannon J, Ernst P, et al. Increased glucocorticoid receptor-beta expression, but not decreased histone deacetylase 2, in severe asthma. J Allergy Clin Immunol 2006;117:703-5.
146. Lu NZ, Cidlowski JA. Translational regulatory mechanisms generate N-terminal glucocorticoid receptor isoforms with unique transcriptional target genes. Mol Cell 2005;18:331-42.
147. Hancox RJ, Stevens DA, Adcock IM, Barnes PJ, Taylor DR. Effects of inhaled beta agonist and corticosteroid treatment on nuclear transcription factors in bronchial mucosa in asthma. Thorax 1999;54:488-92.
148. Hart L, Lim S, Adcock I, Barnes PJ, Chung KF. Effects of inhaled corticosteroid therapy on expression and DNA-binding activity of nuclear factor kappaB in asthma. Am J Respir Crit Care Med 2000; 161:224-31.
149. Kleinert H, Euchenhofer C, Ihrig-Biedert I, Forstermann U. Glucocorticoids inhibit the induction of nitric oxide synthase II by down-regulating cytokine-induced activity of transcription factor nuclear factor-kappa B. Mol Pharmacol 1996;49:15-21.
150. Newton R, Hart LA, Stevens DA, Bergmann M, Donnelly LE, Adcock IM, et al. Effect of dexamethasone on interleukin-1beta-(IL-1beta)-induced nuclear factor-kappaB (NF-kappaB) and kappaB-dependent transcription in epithelial cells. Eur J Biochem 1998;254:81-9.
151. Kim J, Sanders SP, Siekierski ES, Casolaro V, Proud D. Role of NF-kappa B in cytokine production induced from human airway epithelial cells by rhinovirus infection. J Immunol 2000;165:3384-92.
152. Matsukura S, Kokubu F, Kurokawa M, Kawaguchi M, Kuga H, Ieki K, et al. Molecular mechanisms of repression of eotaxin expression with fluticasone propionate in airway epithelial cells. Int Arch Allergy Immunol 2004;134(suppl 1):12-20.
153. Reily MM, Pantoja C, Hu X, Chinenov Y, Rogatsky I. The GRIP1:IRF3 interaction as a target for glucocorticoid receptor-mediated immunosuppression. Embo J 2006;25:108-17.
154. Newton R, Holden NS. Separating transrepression and transactivation: a distressing divorce for the glucocorticoid receptor? Mol Pharmacol 2007;72:799-809.
155. Eddleston J, Herschbach J, Wagelie-Steffen AL, Christiansen SC, Zuraw BL. The anti-inflammatory effect of glucocorticoids is mediated by glucocorticoid-induced leucine zipper in epithelial cells. J Allergy Clin Immunol 2007;119:115-22.
156. Wang JC, Derynck MK, Nonaka DF, Khodabakhsh DB, Haqq C, Yamamoto KR. Chromatin immunoprecipitation (ChIP) scanning identifies primary glucocorticoid receptor target genes. Proc Natl Acad Sci U S A 2004;101:15603-8.
157. Ayroldi E, Migliorati G, Bruscoli S, Marchetti C, Zollo O, Cannarile L, et al. Modulation of T-cell activation by the glucocorticoid-induced leucine zipper factor via inhibition of nuclear factor kappaB. Blood 2001; 98:743-53.
158. Mittelstadt PR, Ashwell JD. Inhibition of AP-1 by the glucocorticoid-inducible protein GILZ. J Biol Chem 2001;276:29603-10.
159. Ayroldi E, Zollo O, Macchiarulo A, Di Marco B, Marchetti C, Riccardi C. Glucocorticoid-induced leucine zipper inhibits the Raf-extracellular signal-regulated kinase pathway by binding to Raf-1. Mol Cell Biol 2002;22:7929-41.
160. Soundararajan R, Zhang TT, Wang J, Vandewalle A, Pearce D. A novel role for glucocorticoid-induced leucine zipper protein in epithelial sodium channel-mediated sodium transport. J Biol Chem 2005;280: 39970-81.
161. Kracht M, Saklatvala J. Transcriptional and post-transcriptional control of gene expression in inflammation. Cytokine 2002;20:91-106.
162. Abraham SM, Clark AR. Dual-specificity phosphatase 1: a critical regulator of innate immune responses. Biochem Soc Trans 2006;34:1018-23.
163. Liu Y, Shepherd EG, Nelin LD. MAPK phosphatases: regulating the immune response. Nat Rev Immunol 2007;7:202-12.
164. Lasa M, Abraham SM, Boucheron C, Saklatvala J, Clark AR. Dexamethasone causes sustained expression of mitogen-activated protein kinase (MAPK) phosphatase 1 and phosphatase-mediated inhibition of MAPK p38. Mol Cell Biol 2002;22:7802-11.
165. Fan J, Yang X, Wang W, Wood WH 3rd, Becker KG, Gorospe M. Global analysis of stress-regulated mRNA turnover by using cDNA arrays. Proc Natl Acad Sci U S A 2002;99:10611-6.
166. Stellato C, Curry SL, Atasoy U, Becker K, Gorospe M, Fan J. Genome-scale analysis of posttranscriptional effects of glucocorticoids (GC). J Allergy Clin Immunol 2006;117:S92.
167. Carrick DM, Lai WS, Blackshear PJ. The tandem CCCH zinc finger protein tristetraprolin and its relevance to cytokine mRNA turnover and arthritis. Arthritis Res Ther 2004;6:248-64.
168. Blackshear PJ. Tristetraprolin and other CCCH tandem zinc-finger proteins in the regulation of mRNA turnover. Biochem Soc Trans 2002;30: 945-52.
169. Carballo E, Lai WS, Blackshear PJ. Feedback inhibition of macrophage tumor necrosis factor-alpha production by tristetraprolin. Science 1998; 281:1001-5.
170. Carballo E, Lai WS, Blackshear PJ. Evidence that tristetraprolin is a physiological regulator of granulocyte-macrophage colony-stimulating factor messenger RNA deadenylation and stability. Blood 2000;95:1891-9.
171. Lai WS, Parker JS, Grissom SF, Stumpo DJ, Blackshear PJ. Novel mRNA targets for tristetraprolin (TTP) identified by global analysis of stabilized transcripts in TTP-deficient fibroblasts. Mol Cell Biol 2006;26:9196-208.
172. Worthington MT, Pelo JW, Sachedina MA, Applegate JL, Arseneau KO, Pizarro TT. RNA binding properties of the AU-rich element-binding recombinant Nup475/TIS11/tristetraprolin protein. J Biol Chem 2002;277:48558-64.
173. Taylor GA, Carballo E, Lee DM, Lai WS, Thompson MJ, Patel DD, et al. A pathogenetic role for TNF alpha in the syndrome of cachexia, arthritis, and autoimmunity resulting from tristetraprolin (TTP) deficiency. Immunity 1996;4:445-54.
174. Pawliczak R, Logun C, Madara P, Barb J, Suffredini AF, Munson PJ, et al. Influence of IFN-gamma on gene expression in normal human bronchial epithelial cells: modulation of IFN-gamma effects by dexamethasone. Physiol Genomics 2005;23:28-45.
175. Edwards MR, Haas J, Panettieri RA Jr, Johnson M, Johnston SL. Corticosteroids and beta2 agonists differentially regulate rhinovirus-induced interleukin-6 via distinct Cis-acting elements. J Biol Chem 2007;282:15366-75.
176. Beck LA, Stellato C, Beall LD, Schall TJ, Leopold D, Bickel CA, et al. Detection of the chemokine RANTES and endothelial adhesion molecules in nasal polyps. J Allergy Clin Immunol 1996;98: 766-80.