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Airway epithelium as an effector of inflammation: molecular regulation of secondary mediators

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Airway epithelium as an effector of inflammation: molecular regulation of secondary mediators. L.D. Martin, L.G. Rochelle, B.M. Fischer, T.M. Krunkosky, K.B. Adler. ©ERS Journals Ltd 1997.
ABSTRACT: Deleterious environmental stimuli cause the airway epithelium to respond with increased secretions of mucus, reaction of oxygen/nitrogen species, changes in ciliary beating, and the influx of inflammatory cells. The epithelium is a target for factors released by infiltrating inflammatory cells, and has recently been shown to serve as an effector of such inflammation. Molecular mechanisms regulating production of secondary inflammatory mediators (cytokines, lipid mediators, and reactive oxygen/nitrogen species) have yet to be fully described.

This report reviews the production of secondary mediators by epithelial cells and by airway epithelium. Lipid mediators are enzymatically produced by the airway epithelium in response to primary mediators. Molecular mechanisms regulating the production of cyclo-oxygenase, lipoxygenase and prostaglandin synthase are discussed, along with the potential of lipid mediators to produce inflammation. The molecular regulation of nitric oxide production is also described in the context of its role as a signalling molecule in pathways regulating secretion of mucus, ciliary motion, and intercellular adhesion molecule-1 (ICAM-1) expression. The production of cytokines by the airway epithelium is shown to play a role in causing inflammation associated with respiratory diseases. Particular attention is paid to molecular mechanisms governing the expression of tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-8 (IL-8).
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Epithelium lining the respiratory airways is the first tissue to encounter deleterious substances, such as airborne pollutants, allergens and microbes. Responses to such stimuli include hypersecretion of mucus, altered ciliary activity, and changes in ion transport/barrier function.

In addition to serving as a target, airway epithelium also serves as an effector, producing inflammatory mediators which act in a paracrine or autocrine fashion to propagate pathophysiological changes. Upon encountering deleterious inhaled stimuli, the epithelium produces primary inflammatory mediators, such as interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α), which, in turn, can provoke production of secondary mediators by epithelial cells, including lipid mediators, cytokines, and reactive oxygen and nitrogen species [1–3]. In many instances, these secondary mediators can affect further pathophysiological alterations (*e.g.* hypersecretion of mucus, increased inflammation) [4, 5]. Thus, secondary mediators appear to play an important role in the pathogenesis of respiratory diseases, such as asthma or chronic bronchitis.

Lipid mediators

Lipid mediators, including prostaglandins, leukotrienes, hydroxyeicosatetraenoic acids (HETEs) and platelet-activating factor (PAF), are prevalent in airways affected by inflammatory disease states, such as asthma. Both infiltrating inflammatory cells, and the airway epithelium itself, produce these lipid mediators in response to a number of stimuli. In turn, these lipid products act both as primary and secondary mediators of airway inflammation.

As primary mediators, PAF and a variety of other cyclo-oxygenase and lipoxygenase products are produced in response to direct environmental stimuli, such as particulates and ozone [6–8]. Exogenous oxidant stimulation of airway epithelial cells *in vitro* also results in production of cyclo-oxygenase products, including prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and thromboxane A_2 (TxA_2) [9, 10].

Production of lipid mediators also occurs in response to a large number of cytokines [11], thus allowing them to serve as secondary mediators of airway inflammation.

These mediators are produced in response to TNF-α and interleukin-1 (IL-1), as demonstrated in a porcine model of adult respiratory distress syndrome [12]. Stimulation of the human bronchial cell line BEAS-2B by TNF-α, INF-γ, and IL-1β also results in rapid activation of prostaglandin G/H synthase-2 transcription and protein expression [13]. In addition, the mast cell product and eosinophil activator, interleukin-5 (IL-5), has been shown to significantly increase the release of 15-HETE from BEAS-2B cells in the presence of calcium ionophore [14]. This release can also be stimulated by IFN-γ [15].

Lipid mediators produced by the epithelium can act in an autocrine or paracrine manner to stimulate the airway epithelium to produce other lipid mediators. For example, PAF, a primary lipid mediator, stimulates the production of eicosanoids, including leukotrienes and HETEs [4, 16]. The bronchoconstrictive effect of PAF has been shown to be dependent on the secondary release of TxA$_2$ [17].

The role of lipid mediators in airway inflammation remains under investigation. They have been shown to be chemotactic for neutrophils, eosinophils and macrophages, to activate eosinophils and macrophages, and to alter vascular and epithelial permeability. Increased expression of the cytokines interleukin-6 (IL-6), TNF-α and INF-γ, by a number of inflammatory cells can occur in response to PAF, leukotriene B$_4$ (LTB$_4$) or prostaglandin E$_2$ (PGE$_2$). These cytokines, in turn, can further exacerbate inflammation due to their chemotactic and gene regulatory activities [3, 18].

Additional manifestations of respiratory disease occur in response to lipid mediators. PAF, leukotrienes, and HETEs can stimulate airway mucin secretion and induce bronchoconstriction [4, 16]. Several of the eicosanoids, including prostaglandin D$_2$ (PGD$_2$), PGE$_2$, and leukotriene C$_4$ (LTC$_4$), have been shown to stimulate epithelial chloride secretion [19]. Chloride secretion is thought to be an important component of epithelial involvement in cystic fibrosis. By contrast, some prostanoids appear to be anti-inflammatory. For example, PGE$_2$ and prostaglandin I$_2$ (PGI$_2$) can counteract the bronchoconstrictor effects of other eicosanoids, and may be major components of epithelial-derived relaxing factor [20, 21].

Molecular mechanisms regulating cyclo-oxygenase and lipoxygenase enzyme production in airway epithelium are of particular therapeutic interest. These enzymes are responsible for the production of lipid mediators from arachidonic acid. Genes coding for lipoxygenase enzymes have been cloned from a number of mammalian sources, including the 5-lipoxygenase gene from an epithelial cell line (HL-60) [22], and the 12-lipoxygenase gene from bovine tracheal epithelial cells [23]. The 15-lipoxygenase pathway is also present in airway epithelial cells [4]. Positive immunofluorescence indicating the presence of the enzyme is observed in basal and ciliated cells from human trachea [24], where messenger ribonucleic acid (mRNA) encoding the enzyme is also found [25]. However, little is known about the function of this pathway in the epithelium.

The promoter region of the 5-lipoxygenase gene contains five tandemly arranged G-C boxes shown to be essential for transcription. There is also evidence for two negative and two positive upstream transcription regulatory elements. However, no potential Ca^{2+} or adenosine triphosphate (ATP)-activation elements are apparent, even though these activities are required for maximal enzyme activity [22, 26]. A number of conserved histidine and glutamine residues have also been shown to be essential for enzymatic structure and activity [27–29]. Studies in Helen Lake (HeLa) cells have shown the enzyme to be translocated to a membrane fraction upon stimulation with calcium ionophore. Experiments suggest that a small "docking" protein (5-lipoxygenase-activating protein (FLAP)) is involved in this translocation [16, 30–32], although its exact role in 5-lipoxygenase activity remains unknown.

The same histidine residues shown to be essential for catalytic activity of 5-lipoxygenase are also essential for 12-lipoxygenase activity. Messenger ribonucleic acid encoding this enzyme is in low abundance (about five copies per cell) in epithelial cells [33]. Two potential sites in the promoter region of the 12-lipoxygenase gene that could account for the induction of transcription by phorbol esters have been noted [34].

The molecular biology of prostaglandin G/H synthase (cyclo-oxygenase) has been examined in tracheal epithelial cells. Complementary deoxyribonucleic acids (cDNAs) encoding this enzyme have been isolated and sequenced from primary ovine tracheal epithelial cells [35], and from the rat tracheal epithelial cell line, EGV-6 [36]. Two interesting features of these mRNAs have emerged. Firstly, there appears to be a multiplicity of transcripts in a single cell type. Two transcripts differing in their 5' ends have been found in EGV-6 cells and in primary rat tracheal epithelial (RTE) cultures. One of these transcripts is improperly spliced. Only the properly processed transcript responds to tissue plasminogen activator (TPA) stimulation in EGV-6 cells, whilst neither transcript responds to TPA-stimulation in the RTE cells [36]. As yet, the significance of this differential processing is unknown. Secondly, the 3'-untranslated region of the ovine prostaglandin G/H synthase-2 gene contains 11 AU-rich elements shown to destabilize mRNAs derived from other genes [35, 37]. The functional significance of these elements has been shown using a bronchial epithelial cell line. With decreasing numbers of AU-rich elements, levels of prostaglandin H (PGH) synthase mRNA rise, suggesting that these elements do serve to destabilize the PGH synthase mRNA [35]. Thus, the molecular regulation of PGH synthase gene expression in airway epithelial cells may prove to occur mainly through post-transcriptional mechanisms.

Reactive oxygen species

Oxidant species are prevalent, at least transiently, in all active sites of metabolism, and are especially important in the pulmonary epithelium, where oxygen and oxidant exposure is continuous. A number of reactive species have been shown to affect cell function, including the oxygen species: superoxide anion radical (O$_2^{\cdot-}$), hydroxyl radical (OH$^\cdot$), hydrogen peroxide (H$_2$O$_2$), and the nitrogen species: nitric oxide (NO$^\cdot$), and peroxynitrite (ONOO$^-$).

Many lesions associated with airway inflammation have been correlated with oxidant stress. Release of oxidants from typical "inflammatory cells," including macrophages, eosinophils and neutrophils, has long been

documented. Production of these species by airway epithelial cells themselves, however, is a relatively recent line of investigation. Bronchial and tracheal epithelial cells release hydrogen peroxide in response to inflammatory stimuli [38, 39]. These reactive oxygen species can be formed through the action of many different cell-associated oxidative enzymes, such as plasma membrane nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH) oxidase and cyclo-oxygenase [40].

Nitric oxide is a unique molecule among the oxidant species, in that it is produced by a small group of specific nitric oxide synthase (NOS) enzymes. The inducible form of NOS (iNOS) has been localized to airway epithelium [41–44], but there is also evidence of constitutive NOS (cNOS) in bronchioles [45]. With the observation that release of nitric oxide from lung and pulmonary alveolar epithelium, following exposure to various cytokines or endotoxin, correlates with an increase in steady-state iNOS mRNA [46, 47], efforts are underway to understand the molecular mechanisms governing iNOS gene expression. Regulation of NOS expression appears to be species- and cell-specific. For instance, synergy between cytokines has been shown for iNOS induction, but the order in terms of strength of induction is different in different cell types. Murine macrophages and human macrophages both express iNOS, but produce very different amounts of NO when induced. It appears that TNF-α induces iNOS but suppresses cNOS expression [48]. In addition, the mode of iNOS regulation may differ depending on the triggering stimulus. For example, macrophage iNOS mRNA appears to be regulated at the level of transcription by lipopolysaccharide (LPS) plus IFN-γ, with no reported changes in mRNA stability [49]. By contrast, transforming growth factor-β (TGF-β) suppresses macrophage iNOS expression by decreasing mRNA and protein stability, but does not alter iNOS transcription [50]. Although there is evidence of NO product inhibition of NOS, phosphorylation may decrease, or increase, activity [7, 51, 52].

Efforts to examine the regulatory region of the iNOS gene have defined at least two regions involved in induction by LPS and IFN-γ. In addition, potential sites for nuclear factor-κB (NF-κB), activating protein-1 (AP-1), nuclear factor-IL-6 (NF-IL-6), interferon response elements, and TNF response elements have been identified [53, 54]. The functional competence of these sites, however, remains under investigation [55].

Reactive oxygen and nitrogen species, known effectors of intracellular signalling in many cell types, are beginning to be recognized as intracellular signalling molecules in airway epithelium. As a signalling molecule, hydroxyl radical has been shown to mediate the secretion of mucus by the airway epithelium following its exposure to reactive oxygen species, such as superoxide, hydrogen peroxide, and ozone [9, 56, 57]. Data from our laboratory also implicate hydroxyl radical as an intracellular signalling molecule for intercellular adhesion molecule-1 (ICAM-1) expression in primary cultures of normal human bronchial epithelial cells and a human bronchial epithelial cell line [58]. The prolonged adherence of infiltrating inflammatory cells to the airway epithelium appears to be dependent on the production of such adhesion molecules.

Nitric oxide (NO) appears to function in the intracellular regulation of secretion of mucus and ciliary motion in airway epithelial cells. NO has been shown to upregulate stimulated ciliary beating in bovine bronchial epithelial cells [59]. Studies from our laboratory demonstrate that inhibition of intracellular NO production blocks hypersecretion of mucin in guinea-pig tracheal epithelial cells stimulated by reactive oxygen species, TNF-α, PAF, or histamine [60–62]. Therefore, nitric oxide appears to be produced by the airway epithelium in response to primary inflammatory mediators, such as TNF-α and PAF, possibly serving to signal the upregulation of cellular processes that increase the inflammation observed in respiratory disease. Thus, nitric oxide may be considered a secondary mediator of inflammation produced by the effector tissue, the airway epithelium. Nitric oxide also has the potential to react quickly with the large quantities of superoxide and protein thiols available to form peroxynitrite or nitrosothiols [63], which may also serve as secondary mediators of inflammation.

In addition to serving as signalling molecules to promote inflammation, oxidant species can directly injure the airway, prompting further infiltration of inflammatory cells. In this manner, reactive oxygen/nitrogen species produced by inflammatory cells and the airway epithelium can alter the expression and activation of oxidant-regulated transcription factors, such as NF-κB and AP-1 [40], many of which are involved in regulating the expression of proinflammatory cytokines, such as interleukin-8 (IL-8) and IL-6.

Cytokines

Pluripotent cytokines produced by the airway epithelium play an important role in inducing airway inflammation. Primary mediators, including the cytokines, IFN-γ and TNF-α, upregulate production of secondary mediators produced by the airway epithelium. These secondary mediators include IL-6, IL-8, granulocyte/macrophage colony-stimulating factor (GM-CSF), and insulin-like growth factor-1 (IGF-1).

TNF-α

TNF-α, originally identified as a product of activated macrophages, is now known to be produced by many resident airway cells, including alveolar macrophages, endothelial and mast cells, leucocytes and the airway epithelium [3, 64]. TNF-α has been shown to have multiple biological effects and has been implicated in airway diseases, including asthma and pulmonary fibrosis [60, 65–67].

Whilst the ability of TNF-α to serve as a primary mediator of inflammation in airway epithelial cells is well-documented, the signal transduction mechanisms involved in regulating TNF-α synthesis and secretion in these cells is still largely unknown. However, investigations in renal epithelial cells, macrophages, and the epithelial cell line HL-60, demonstrate that TNF-α secretion can be stimulated with IL-1β, TNF-α, endotoxin, calcium flux, oxygen free-radical mechanisms and phorbol esters [67–69]. In HL-60 cells, products from the 5-lipoxygenase

pathway, 5-hydroperoxyeicosatetraenoic acid and leukotriene B$_4$ have been shown to increase TNF-α transcripts, leukotrienes, at least in part, by increasing the rate of TNF-α transcription [69]. TNF-α can also induce its own expression at the ribonucleic acid (RNA) and protein level, an effect associated with an increase in the rate of TNF transcription [17, 70]. Inhibitors of phospholipase A$_2$ can block this induction [69]. Since other inflammatory mediators, such as leukotrienes and TNF-α itself, can stimulate TNF-α gene expression, TNF-α can be said to serve as a primary and secondary mediator of airway inflammation.

Comparison of the 5'-flanking region of the TNF-α gene from various sources has revealed conserved, potential regulatory sites, including consensus sequences for the AP-1 and activating protein-2 (AP-2) sites, the cyclic adenosine monophosphate (cAMP)-responsive element, and sequences similar to the kappa B sequences found in immunoglobulin and cytokine regulatory elements. A decanucleotide resembling the "Y-box" of major histocompatibility (MHC) class II promoters is also located in this region [67, 69].

The functional importance of most of these regulatory sites remains undetermined. However, a TPA-responsive element has been located in the TNF-α promoter region using reporter gene constructs transfected into HeLa cells. Similarly, a binding site for the phorbol ester responsive transcription factor, AP-2, has been located [71].

In addition to conserved upstream elements, the 3'-untranslated region of the TNF-α gene contains a conserved sequence element, which has been associated with accelerated degradation of mRNA and translational interference [72]. Using reporter gene constructs, it has been demonstrated that this sequence, present near the TNF-α gene, is sufficient to induce synthesis in response to endotoxin. This effect appears to be due to an enhancement of translational efficiency [73].

Once secreted, TNF-α yields numerous inflammatory effects following binding to two distinct membrane receptors [74, 75]. In airway epithelial cells, for example, TNF-α has been shown to alter cell migration and permeability, and to stimulate IL-6, IL-8 and GM-CSF secretion [3, 76]. These potent cytokines, in turn, can exert their biological effect on cells within the local environment, including interstitial fibroblasts, infiltrated leucocytes and pulmonary endothelium, to enhance the inflammatory state. For example, TNF-α stimulates expression of adhesion molecules, such as ICAM-1 and vascular adhesion molecule-1 (VCAM-1), on pulmonary endothelium and airway epithelium. These adhesion molecules signal infiltration of leucocytes into the lung. TNF-α also stimulates endothelial leucocyte adhesion molecule (ELAM-1) and lymphocyte function-associated antigen-1 (LFA-1) on eosinophils and neutrophils, further enhancing margination and diapedesis resulting in airway inflammation [77].

Secondary cytokines as effectors of inflammation

IL-6 and IL-8 are among the many secondary mediators produced by airway epithelium in response to primary inflammatory mediators. A number of studies have noted the presence of these cytokines in disease states, such as asthma and cystic fibrosis [78, 79]. One study, comparing bronchial epithelial cells isolated from asthmatic patients with those from healthy control subjects, found GM-CSF, IL-6 and IL-8 protein and mRNA in virtually all samples from asthmatic individuals, whilst little to none were found in control samples. In contrast to healthy cells, the asthmatic cells also released these cytokines in culture [79].

As airway epithelial cells synthesize and release proinflammatory secondary mediators, the migration of granulocytes and lymphocytes into this tissue occurs, along with local activation of these cells [80–82]. Specifically, IL-6 induces T-cell activation and proliferation [82, 83], whilst augmenting immunoglobulin production by B-lymphocytes [84]. IL-8 is chemotactic for neutrophils, eosinophils, basophils and T-lymphocytes [85]. GM-CSF activates eosinophils and prolongs their survival in asthmatic airways.

In addition to their proinflammatory effects, some cytokines can affect airway epithelium directly. For example, exposure of human bronchial explants to 20 ng·mL^{-1} IL-6 increases secretion of mucus. Since an early increase in secretion following administration of IL-6 was not accompanied by an increase in mRNA, it is possible that IL-6 triggers the exocytosis of preformed mucus. IL-6 has also been shown to increase the level of steady-state mRNA of the mucin 2 (*MUC2*) gene in normal human airway epithelial cells [86]. IL-6 induces respiratory mucous glycoprotein secretion and *MUC2* gene expression by human airway epithelial cells. In cystic fibrosis, elastase produced by resident neutrophils in airways induces the bronchial epithelium to secrete IL-8, which in turn recruits additional neutrophils, which self-perpetuates the inflammatory process [87].

Molecular regulation of IL-8

Few studies have investigated the molecular regulation of cytokines produced by airway epithelium. It has been shown using the pulmonary type II-like epithelial cell line A549, that IL-8 steady-state mRNA increases in a time- and dose-dependent fashion in response to TNF-α, IL-1α or IL-1β [85]. IL-8 transcription can also be increased with phorbol ester [86]. Similar increases have been noted in primary cultures of human bronchial epithelial cells [1]. In these cells, neutrophil elastase has also been shown to increase the steady-state level of IL-8 mRNA in a dose- and time-dependent manner. This induction occurs due to an increase in IL-8 transcription, with no changes in message stability [87]. In contrast, glucocorticoids do not appear to have a significant effect on the regulation of IL-8 gene expression in airway epithelial cells (BEAS-2B) [86].

Recently, a role for oxidants in the regulation of IL-8 expression in airway epithelium has been discovered. Oxidant stress was found to stimulate IL-8 production in a variety of cell lines, including A549 [88]. Antioxidants have also been shown to decrease IL-8 release and mRNA in a time- and dose-dependent fashion in cells challenged with respiratory syncytial virus. In contrast, inhibitors of the specific oxidant nitric oxide had no effect on IL-8 gene expression [89]. The promoter region of the IL-8 gene contains a binding site

for NF-κB, which may play a role in IL-8 transcription, since proteins in nuclear extracts from cells stimulated with LPS bind to a synthetic oligo corresponding to the NF-κB element present upstream of the IL-8 gene. The antioxidant, N-acetylcysteine, can block this binding, further suggesting that oxidative changes which affect the activation of NF-κB are involved in the regulation of IL-8 gene expression [90].

An NF-IL-6 binding site is also present in the IL-8 promoter, and, whilst its importance in regulating IL-8 expression in response to nitric oxide has been demonstrated in a human melanoma cell line, no such experimentation has been undertaken in airway epithelium [91].

Molecular regulation of IL-6

Whilst little is known directly about the regulation of IL-6 in airway epithelium, much is known about its regulated expression in other tissue types [92–94], and in less well-differentiated epithelial cell lines (HeLa cells) [95]. Molecular dissection of the IL-6 promoter has revealed its potential responsiveness to at least three signal transduction pathways: protein kinase C; cAMP/protein kinase A; and calcium ionophore. Work in HeLa cells has shown that 225 base pairs (bp) of the IL-6 5'-flanking sequence are sufficient to confer a transcriptional response to agents that may invoke these pathways, including IL-1, TNF-α, phorbol ester, and serum [95]. The function of this IL-6 promoter is of great interest, since it responds to various stimuli through a single 23 bp element, activating multiple signal transduction pathways [96].

Sequence analysis, coupled with mutational analysis, has revealed a number of functionally important elements within the 225 bps upstream of the IL-6 gene. Two partially overlapping deoxyribonucleic acid (DNA) response elements (MRE I and II) compose the multiple cytokine (IL-1, TNF, serum) and second messenger (cAMP, phorbol ester)-responsive enhancer region. The MRE II element is composed of an imperfect dyad repeat, and contains a binding site for NF-IL-6. The second responsive element (MRE I) contains an AP-1-like site. Mutations in the AP-1 site block induction by cAMP or phorbol ester, but not by IL-1 and TNF. Mutation of the NF-IL-6 site greatly reduces the responsiveness of the promoter to IL-1. Complete loss of IL-1 responsiveness occurs with mutation of the NF-κB site in the promoter region [97, 98]. Mutation of the NF-κB site also decreased induction of the IL-6 promoter to stimulation by rhinovirus in A549 cells [99]. A 21 out of 26 nucleotide match with the retinoblastoma susceptibility gene product element is also located within the promoter region of the IL-6 gene [97, 98]. While the function of this element in regulating IL-6 gene expression has not been directly demonstrated, it is known that overexpression of the wild-type human retinoblastoma susceptibility gene product represses serum-induced IL-6 expression in HeLa cells [100].

Preliminary results from our laboratory, using primary human bronchial epithelial cells, indicate an upregulation of IL-6 steady-state mRNA in response to TNF-α stimulation. In addition, a similar increase in IL-6 mRNA was noted when cells were exogenously exposed to the superoxide-generating system purine + xanthine oxidase, or to a reduction in nitric oxide due to the presence of the nitric oxide inhibitor, L-N^6-monomethylargenine (LNMA) (Martin and Adler, unpublished data). Since NF-κB is also activated in these cells following stimulation with TNF-α (Krunkosky and Adler, unpublished data), the potential for activating IL-6 expression *via* a more direct oxidative pathway also exists.

IL-6 gene expression can also be decreased by treatment with corticosteroids, including dexamethasone [86]. While the precise mechanism of this downregulation has not been determined in airway epithelium, in HeLa cells, downregulation is due to binding of the ligand-activated glucocorticoid receptor to the multiple cytokine and second-messenger responsive elements, and the transcriptional regulatory regions of the IL-6 promoter [101].

Summary

As the first tissue to encounter the external environment, the human airway epithelium has evolved a number of protective mechanisms. As a target, it responds to external allergens, pollutants and oxidants through a variety of mechanisms, including the production of mucus, changes in ciliary beating, production of, and reaction with, oxygen/nitrogen reactive species. Cellular reactions to these external stimuli can include activation and infiltration of inflammatory cells to the affected airway. The airway must then respond to these cells, and mediating substances produced by them. In addition, the airway epithelium acts as an effector, producing additional secondary mediators, such as lipids and cytokines, which can have paracrine and autocrine effects on the epithelium and its surrounding tissues and cells. Whilst much is known about the signal transduction mechanisms and some of the molecular mechanisms activating the production of inflammatory mediators by the infiltrating cells, far less is understood about the production of such mediators by, and their effects on, the airway epithelium, especially at the molecular level. As one of the next greatest challenges in pulmonary biology, dissecting the molecular regulation and action of these mediators should provide new avenues for development of therapeutics, including gene therapies, for use in a variety of airway diseases.

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