Role of Airway Epithelium in Airway Inflammation

Kenneth B. Adler

North Carolina State University, College of Veterinary Medicine, Raleigh, NC 27606

Introduction

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, and altered ciliary activity and 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 pathophysiologic changes. Upon encountering deleterious inhaled stimuli, the epithelium begins producing primary inflammatory mediators such as interferon gamma and TNFa, which in turn can provoke production of secondary mediators by epithelial cells, including lipid mediators, cytokines, and reactive oxygen and nitrogen species. In many instances, these mediators can affect further pathophysiological alterations (e.g. hypersecretion of mucus, increased inflammation). Thus, secondary mediators could play an important role in the pathogenesis of respiratory diseases, such as asthma or 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. These lipid mediators are produced by infiltrating inflammatory cells, but also by airway epithelium. Environmental stimuli, such as particulates and ozone, provoke production of PAF and a variety of cycloxygenase and lipoxygenase products. Oxidant stress on airway epithelial cells in vitro results in production of cycloxygenase products including PGF2a and thromboxane A2. TNFa and IL-1 provoke production of similar lipid mediators, and the mast cell product and eosinophil activator, interleukin 5 (IL-5), increases release of 15-HETE by the human bronchial epithelial cells. Release of 15-HETE also can be stimulated by interferon-gamma through a mechanism involving cytosolic phospholipase A2. Epithelial derived lipid mediators also can act in an autocrine or paracrine manner to stimulate airway epithelium to produce other lipid mediators. For example, PAF stimulates eicosanoid production by airway epithelium.

Much is known about the signal transduction pathways governing production, release, and effects of secondary lipid mediators. PAF stimulates mucin secretion through a pertussis toxin-sensitive G protein and by a protein kinase C dependent mechanism. Both PAF and 15-HETE appear to activate phospholipase C.

In addition, work has begun on the molecular mechanism regulating cycloxygenase and lipoxygenase enzymes which play a role in metabolizing arachidonic acid to produce

epithelial lipid mediators. In a rat tracheal epithelial cells, phorbol esters (which are known activators of protein kinase C) induce functional prostaglandin G/H synthase-1 gene expression, a process inhibitable by glucocorticoids and requiring de novo RNA and protein synthesis. Cytokines, such as TNFa, can upregulate message levels of cytosolic phospholipase A2 in human airway epithelium.

The specific contribution(s) of these lipid mediators to inflammation remains under investigation. They have been shown to be chemotactic for neutrophils and eosinophils, to activate eosinophils, and to alter vascular and epithelial permeability and to increase airway mucin secretion. PAF, leukotrienes, and HETEs all can stimulate airway mucin secretion, induce bronchoconstriction, and function as chemoattractants or activators of leukocytes and macrophages.

Reactive Oxygen Species

Reactive oxygen and nitrogen species can be involved in intracellular or extracellular reactions and have been shown to alter many cell components including DNA, lipids, enzyme active sites, and receptors. In addition, reactive oxygen species can activate the transcription factors NF-kB and AP-1. Oxidant species are prevalent, at least transiently, in all active sites of metabolism, and are especially important in pulmonary epithelium where oxygen and oxidant exposure is continuous. Several reactive species have been shown to affect cell function including reactive oxygen species: superoxide anion, hydroxyl radical, hydrogen peroxide; and reactive nitrogen species: nitric oxide, and peroxynitrite.

Many lesions associated with airway inflammation may be related to oxidant stress. Release of oxidants in the airways 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 have been shown to release hydrogen peroxide in response to inflammatory stimuli.

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 has been localized to airway epithelium, but there is also evidence of a constitutive NOS in human airways. Endogenously produced NO has a role in mucus secretion and ciliary beating.

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, efforts are underway to understand the molecular mechanisms governing iNOS gene expression. Regulation of NOS expression appears to be species and cell-specific. Synergy between cytokines has been shown for iNOS induction, but the strength of induction is differs in different cell types. Murine macrophages and human macrophages both express iNOS, but produce very different amounts of NO when induced. In addition, the mode of iNOS regulation may differ depending on the triggering stimulus. Macrophage iNOS mRNA appears to be regulated at the level of transcription by LPS plus IFNg, with no reported changes in mRNA stability. In contrast, TGFb suppresses macrophage iNOS expression by decreasing

mRNA and protein stability, but does not alter iNOS transcription. Although there is evidence of NO product inhibition of NOS, phosphorylation may decrease or increase activity.

Efforts to examine the regulatory region of the iNOS gene have defined at least two regions involved in induction by LPS and IFNg. Potential sites for NF-kB, AP-1, NF-IL-6, interferon response elements, and TNF response elements have also been identified. The functional competence of these sites, however, remains under investigation.

Thus, airway inflammation in diseased patients may be exacerbated by reactive oxygen species. Such reactive species may cause further injury to the airway, prompting infiltration of "inflammatory cells." Indirectly, reactive oxygen/nitrogen species alter the expression and activation of oxidant-regulated transcription factors, many of which are involved in regulating the expression of proinflammatory cytokines such as IL-8 and IL-6.

Cytokines

Pluripotent cytokines produced by the airway epithelium play an important role in inducing airway inflammation. These cytokines include IFNg and TNFa, which, as primary mediators, upregulate production of secondary mediators produced by airway epithelium, including interleukin 6 (IL-6), interleukin 8 (IL-8), granulocyte-macrophage colony stimulating factor (GM-CSF), and insulin-like growth factor (IGF-1).

TNFa

TNFa, originally identified as a product of activated macrophages, is now known to be produced by many resident airway cells including alveolar macrophages, endothelium, mast cells, leukocytes and airway epithelium. TNFa has been shown to have multiple biological effects and has been implicated in airway diseases including asthma and pulmonary fibrosis.

Mechanisms regulating TNFa synthesis and secretion in airway epithelial cells are still largely unknown. However, investigations of these mechanisms in renal epithelial cells and macrophages demonstrate that TNFa secretion can be stimulated with IL-1 b, endotoxin, calcium, oxygen free radical mechanisms, and the phorbol ester PMA. Research investigating the 5'flanking region of the TNFa gene has revealed multiple potential regulatory sites, including consensus sequences for the AP-1 and AP-2 sites, the cAMP-responsive element, and similar sequences to the kappa B sequences found in immunoglobulin and cytokine regulatory elements. The importance and interactions of these regulatory sites, however, remain undetermined.

Once secreted, TNFa initiates numerous inflammatory effects. In airway epithelial cells, TNFa has been shown to alter cell migration and permeability, and to stimulate IL-6, IL-8, and GM-CSF secretion. These potent cytokines, in turn, can exert their biological effects on cells within the local environment, including interstitial fibroblasts, infiltrated leukocytes, and pulmonary endothelium, and thus may enhance the inflammatory state. For example, TNFa stimulates expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1(VCAM-

1) on pulmonary endothelium and airway epithelium. These adhesion molecules regulate infiltration of leukocytes into the lung. TNFa also stimulates endothelial leukocyte 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.

Secondary cytokines as effectors of inflammation

IL-6 and IL-8 are among the many secondary mediators produced by airway epithelium. A number of studies have noted the presence of these cytokines in disease states such as asthma and cystic fibrosis. 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, while little to none were found in control samples. The asthmatic cells also released these cytokines in culture.

As airway epithelial cells synthesize and release proinflammatory secondary mediators such as IL-6, GM-CSF, and IL-8, the migration of granulocytes and lymphocytes into this tissue occurs, along with local activation of these cells. Specifically, IL-6 induces T cell activation and proliferation while augmenting immunoglobulin production by B lymphocytes. IL-8 is chemotactic for neutrophils, eosinophils, basophils and T lymphocytes, while GM-CSF activates eosinophils and prolongs their survival in asthmatic airways. Thus, the airway epithelium can be viewed as a signaling device, sending a clear message to the inflammatory and immune system that penetration or disruption of the airway mucosa has occurred.

In addition to their proinflammatory effects, some cytokines can affect airway epithelium directly. For example, exposure of human bronchial explants to 20 ng/ml IL-6 increases mucus secretion. Since an early increase in secretion following administration of IL-6 was not accompanied by an increase in messenger RNA, it is possible that IL-6 triggers the exocytosis of preformed mucus. By contrast, IL-6 has been shown to increase the level of steady-state mRNA of the mucin 2 gene (MUC2) in human airway epithelial cells obtained from bronchial brushings. IL-6 induces respiratory mucous glycoprotein secretion and MUC-2 gene expression by human airway epithelial cells.

Molecular Regulation of IL-8

Few studies have delved into 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 TNFa, IL-1a or IL-1b. Similar increases have also been noted in primary cultures of human bronchial epithelial cells. IL-8 transcription can also be stimulated with the Protein Kinase C activator, PMA. In contrast, glucocorticoids do not appear to have a significant effect on regulation of IL-8 gene expression in airway epithelial cells.

Recently, a role for oxidants in regulation of IL-8 expression in airway epithelium was suggested. Oxidant stress was found to stimulate IL-8 production in a variety of lung epithelial cell lines. Similar effects were found when these cells were infected with respiratory syncytial virus; antioxidants could decrease IL-8 release and mRNA in a time-

and dose-dependent fashion. In contrast, inhibitors of nitric oxide, a specific oxidant, had no effect on IL-8 gene expression. The promoter region of the IL-8 gene contains a binding site for NF-kB which is likely necessary for IL-8 transcription, since protein binds to the element after stimulation of cells with LPS. The antioxidant N-acetyl cysteine can block this binding, suggesting a role for oxidative changes in regulation of IL-8 gene expression. An NF-IL-6 binding site is also present in the IL-8 promoter, and, while 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.

Molecular Regulation of IL-6

While little is known directly about the regulation of IL-6 in airway epithelium, much is known about its regulated expression in other tissue types (such as keratinocytes, osteoblasts and fibroblasts) and in less well-differentiated epithelial cell lines (eg. HeLa cells). The production of IL-6 can be upregulated by TNFa through an increase in steady-state IL-6 messenger RNA in human bronchial epithelial cells. The multiple cytokine (IL-1, TNF, serum)-and second messenger (cAMP, phorbol ester)-responsive enhancer region of the IL-6 promoter is composed of two partially overlapping DNA elements. The region from -145 to -158 (the MRE II site) is composed of an imperfect dyad repeat, and contains a binding site for nuclear factor IL-6 (NF-IL-6). This region is partially overlapped by a second responsive element (MRE I) which contains an AP-1-like site. Both elements strongly respond to induction by phorbol esters and protein kinase A agonists, as well as to cytokine stimulation with IL-1 and TNF.

Molecular dissection of the IL-6 promoter reveals its potential responsiveness to activation by at least three signal transduction pathways--protein kinase C, cAMP/protein kinase A, and calcium ionophore. Mutation of MRE I at the AP-1 site results in a block to induction by cAMP or phorbol ester, but not by IL-1 or TNF. Mutation of the NF-IL-6 site in MRE II greatly reduces the responsiveness of the promoter to IL-1. Complete loss of IL-1 responsiveness occurs with mutation of the NF-kB site. Therefore, the efficient induction of the IL-6 promoter with subsequent production of IL-6 protein in tissues such as airway epithelium appears to depend on the cooperative interactions of several transcription factors including members of the NF-IL-6 or C/DBP family, AP-1 and NF-kB. Preliminary results from our laboratory using primary human bronchial epithelial cells indicate an upregulation of IL-6 steady-state messenger RNA in response to exogenous oxidant stress or inhibition of NOS. Since NF-kB is activated in these cells following stimulation with TNFa, the potential for activating IL-6 expression via a more direct oxidative pathway also exists.

Conclusion

As the first tissue to encounter the external environment, airway epithelium has evolved a number of protective mechanisms. As a target, it responds to external allergens, pollutants, and microbes through a variety of protective mechanisms including production of mucus, changes in ciliary beating, changes in barrier function, and production of reactive species. In addition, the airway epithelium can act an

inflammatory "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. While much is known about the signal transduction mechanisms and some of the molecular mechanisms activating production of inflammatory mediators by infiltrating cells, far less is understood about production of such mediators by, and their effects on, the airway epithelium, especially at the molecular level. One of the most important areas in pulmonary biology in the future relates to elucidating the molecular regulation and action of these mediators. This may provide new avenues for development of therapeutic approaches, including gene therapies, for use to treat a variety of airway diseases.