

Updates on cells and cytokines

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Epithelial cells as regulators of airway inflammation

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The past decade has seen major advances in our understanding of the metabolic and biochemical functions of the epithelial cell. As a result, the traditional view of the epithelium as a relatively passive physical barrier to the external environment has been superseded by the concept that the epithelial cell plays a key role in regulating airway inflammation. The epithelial cell exerts this modulatory effect in several ways. Epithelial cells can function to maintain mucosal integrity and to modulate local immune responses. They can also limit inflammatory processes by degrading, or inhibiting, proinflammatory mediators and proteins. However, the epithelium also responds to a range of stimuli by producing biologically active mediators that can influence airway inflammation. These include, but are not limited to, a broad range of cytokines and chemokines that can exert profound effects on inflammatory cells, as well as lipid and peptide mediators. This review will highlight some of these aspects of the role of the epithelium in regulating airway inflammation. (*J Allergy Clin Immunol* 1998;102:714-8.)

In the past decade the classical view of the epithelium as a physical barrier between the host and its environment has rapidly been replaced by a more complex description in which epithelial cells also act as central modulators of the inflammatory response.¹ This modulatory role encompasses both "target" and "effector" type responses, both of which are important in asthma and related allergic diseases. In a "target" capacity, the influence of stimuli on the epithelial layer results in altered modulation of airway secretions, mucociliary clearance, and ion transport. It is the more recently appreciated "effector" role of the epithelium, however, that will be the focus of this review. We will briefly summarize the capacity of epithelial cells to respond to exogenous and endogenous stimuli by generating a range of biologically active mediators, by participating in immune regulation, and by helping to maintain mucosal integrity.

Abbreviations used

BAL:	Bronchoalveolar lavage
MCP:	Monocyte chemoattractant protein
NEP:	Neutral metalloendopeptidase 24.11
NO:	Nitric oxide
NOS:	Nitric oxide synthase
PAF:	Platelet-activating factor
RANTES:	Regulated on activation, normal T-cell expressed and secreted
TGF:	Transforming growth factor

MEDIATOR GENERATION

Among the several classes of molecules produced by the epithelium, we will focus on cytokines and chemokines, low molecular weight lipid and peptide mediators, reactive oxygen species, and catabolic enzymes and enzyme inhibitors.

Cytokines

Of the mediators produced by epithelial cells, none have received as much recent attention as the cytokines. Epithelial production of cytokines may lead to the recruitment and activation of inflammatory cells to the airway during inflammatory diseases (Fig 1). The cytokines actively synthesized and/or released by the epithelium can be grouped into several main categories, including colony-stimulating factors, pleiotropic cytokines, chemoattractant cytokines, and growth factors (Table I).

GM-CSF was the first of the colony-stimulating factors detected as a product of epithelial cells and has since been shown to be constitutively synthesized and released by airway epithelial cells. It has been proposed as a potential mediator of asthmatic inflammation because its spectrum of biologic activity includes prolongation of eosinophil survival and enhancement of the cytotoxic activity, mediator release, and phagocytic ability of neutrophils, eosinophils, and macrophages. Indeed, expression of GM-CSF is increased in bronchial biopsy specimens from asthmatic patients. Other colony-stimulating factors, including G-CSF, M-CSF, and CSF-1, have also been established as epithelial cell products.

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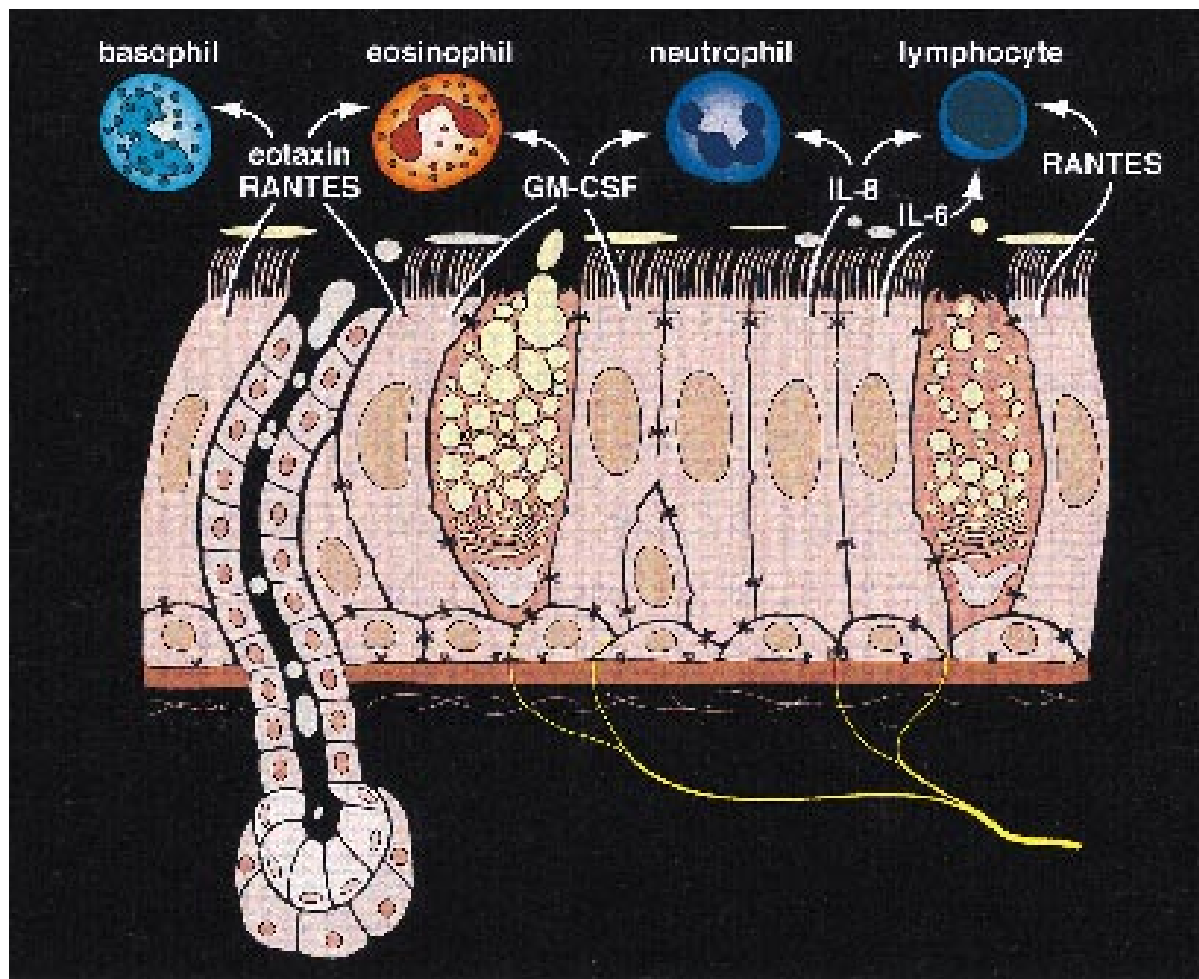


FIG 1. The epithelium produces cytokines that can recruit and activate inflammatory cells. The epithelium responds to a range of stimuli by producing chemoattractant cytokines, such as IL-8, eotaxin, and RANTES, that can recruit inflammatory cells to the airways. The production of colony-stimulating factors, such as GM-CSF, and of pleiotropic cytokines, such as IL-6, can further modulate the activation state of these cells—see text for details (© 1997 Jacqueline Schaffer).

Among the epithelial-derived pleiotropic cytokines, IL-6 and IL-11 are the best studied. Increased IL-6 gene expression has been observed in epithelial cells from bronchial biopsy specimens of asthmatic subjects, and increased IL-6 levels are found in the bronchoalveolar lavage (BAL) fluid of symptomatic asthmatic subjects. Transgenic mice that overexpress IL-6 in airway epithelial cells are hyporesponsive to methacholine, leading to the suggestion that IL-6 production might be an attempt by the host to normalize a dysfunctional airway.² IL-11, a cytokine with some similarities to IL-6, is also produced by epithelial cells, particularly when they are stimulated by IL-1, transforming growth factor (TGF)- β , or respiratory viruses. Elevated levels of IL-11 are detected in nasal secretions of children with respiratory infections, with the highest levels being found in subjects with audible wheezing. Interestingly, provocation of mice with IL-11 induces airways hyperresponsiveness, whereas targeted overexpression in the airways leads to airway remod-

eling. Epithelial cells have also been reported to produce low levels of other pleiotropic cytokines, including IL-1, IL-10, and TNF- α , under some conditions.

The chemoattractant cytokines, or chemokines, are a superfamily of secreted molecules that have been increasingly recognized to play an important role in allergy and inflammation. The 2 main groups of chemokines are designated as the C-X-C or α family, and the C-C or β family. Given its anatomical location, production of chemokines by the epithelium could play a pivotal role in recruiting inflammatory cells to the airways during allergic diseases. Among the C-X-C chemokines, IL-8 is produced in large amounts by cultured human epithelial cells. Increased expression of IL-8 is seen in bronchial epithelial cells of asthmatic subjects and in nasal epithelial cells of allergic subjects in season. This may be relevant to disease pathogenesis because IL-8 is chemotactic for neutrophils, some subsets of T lymphocytes, and activated eosinophils. GRO- α

TABLE I. Epithelial-derived cytokines

Colony-stimulating factors	Pleiotropic cytokines	Growth factors	Receptors/antagonists	Chemoattractant cytokines		
				Lymphocyte chemoattractant factor	C-X-C/ α chemokines	C-C/ β chemokines
GM-CSF	IL-6	TGF- β	Type 1 TNFR	IL-16	IL-8	RANTES
G-CSF	IL-11	TGF- α	icIL-1Ra Type 1		GRO- α	MCP-1
M-CSF	IL-1	SCF			GRO- γ	MCP-4
CSF-1	IL-10	bFGF				Eotaxin
	TNF- α					MIP-1 α (rat)

SCF, Stem cell factor; bFGF, basic fibroblast growth factor.

and GRO- β , which are chemotactic for neutrophils, are 2 other members of the C-X-C family that are produced by human airway epithelium.

The epithelium also produces C-C chemokines that could recruit eosinophils to the airways during allergic diseases. These include regulated on activation, normal T-cell expressed and secreted (RANTES); eotaxin; and monocyte chemoattractant protein (MCP)-4. Basal production of RANTES or eotaxin by the epithelium is minimal, but it increases on exposure to stimuli such as respiratory viruses, TNF- α , or IFN- γ . Epithelial expression of eotaxin has been shown to be increased in chronic sinusitis, whereas RANTES expression is increased in nasal polyps. In the lung, levels of eotaxin and RANTES are increased in BAL fluids from asthmatic subjects, and it has been reported that RANTES is among the most important eosinophil chemoattractants in the asthmatic lung.³

IL-16 is a chemoattractant cytokine that does not belong to either the C-X-C or C-C chemokine families. It functions as a chemoattractant for CD4⁺ cells, including T-helper lymphocytes and eosinophils, and may therefore contribute to the characteristic airway mucosal infiltration by CD4⁺ cells in asthma. IL-16 is produced by epithelial cells and is present in increased amounts in bronchial epithelial cells of subjects with atopic asthma,⁴ as well as in BAL fluid from antigen-challenged asthmatic subjects.

The production of growth factors from the epithelium has not been as intensively studied, but it has been reported that TGF- α and TGF- β , as well as stem cell factor and basic fibroblast growth factor, are produced by epithelial cells.

Lipid mediators

Human airway epithelial cells have the ability to convert arachidonic acid to a host of lipid mediators of inflammation.⁵ The 15-lipoxygenase pathway predominates and converts arachidonic acid to 15-hydroxy-eicosatetraenoic acid, as well as to a broad range of biologically active hydroperoxy, epoxyhydroxy, keto, and dihydroxy acids. Airway inflammation induces 15-lipoxygenase activity, with increased levels being observed in the airway epithelium of subjects with asthma and chronic bronchitis. It is minimally present in the

airway epithelium of normal subjects, being confined to the nose and trachea. The major metabolite, 15-hydroxy-eicosatetraenoic acid, further modulates inflammation through a variety of secondary effects, including activation of 5-lipoxygenase in mast cells, secretion of mucus glycoprotein by the airway, and augmentation of the early response to allergen challenge.

Arachidonic acid is also metabolized in airway epithelial cells by the cyclooxygenase pathway. Nearly equal amounts of PGE₂ and PGF_{2 α} are made by the epithelium. It has been reported that PGE₂ is protective against the bronchoconstrictor effects of antigen challenge, presumably because of its bronchodilator properties. Although the lipoxygenase pathway often predominates in epithelial cells, the preferred route of metabolism may depend on the available arachidonic acid concentration because maximal cyclooxygenase activity occurs at lower substrate concentrations than for the lipoxygenases.

Another lipid mediator, platelet-activating factor (PAF), is made in small amounts by human bronchial epithelial cells. PAF could contribute to the inflammatory response in the lung because its effects include neutrophil and eosinophil recruitment and increased vascular permeability.

Peptide mediators

Among the peptide mediators produced by the epithelium, the endothelin family has been best characterized. Endothelin-1, endothelin-2, and endothelin-3 are closely related and function as potent constrictors of vascular and airway smooth muscle. Increased levels of endothelin-1 and endothelin-3 have been detected in the BAL fluid of asthmatic subjects, and levels paralleled the severity of symptoms.⁶ Endothelin can also stimulate mucus production and smooth muscle proliferation and may serve in an autocrine regulatory capacity by stimulating release of lipid mediators from the epithelium.

Other peptide products, including vasopressin, substance P, and calcitonin gene-related peptide have been reported as part of the spectrum of molecules secreted by the epithelium, but they are less well studied. The recent finding of epithelial expression of human β -defensin-1 is particularly interesting because this may play a role in the mucosal defenses of the lung through its broad antimicrobial activity.⁷

Reactive oxygen species

Although macrophages, eosinophils, and neutrophils have traditionally been viewed as the major sources of reactive oxygen species, epithelial cells are now known to produce these mediators as well. Guinea pig tracheal and bovine bronchial epithelial cells release hydrogen peroxide after stimulation with PAF or phorbol myristate acetate. The amount of hydrogen peroxide released by individual epithelial cells is small (approximately 100-fold less than that derived from macrophages), but when taken as a whole, the entire respiratory tract can be a significant source of this molecule. Hydrogen peroxide may also have an autocrine effect on the epithelium through its stimulation of IL-8 production.

Recently, considerable attention has focused on the ability of the epithelial cell to produce nitric oxide (NO), a signaling molecule with vasodilator, bronchodilator, and immunomodulatory effects. Although it has been reported that epithelial cells express all 3 isoforms of the enzyme NO synthase (NOS), it is likely that NO generation by the epithelium occurs predominantly through the actions of inducible NOS, also called NOS-2. Expression of inducible NOS is clearly increased in the airway epithelium of subjects with asthma compared with nonasthmatic control subjects, and levels of NO in exhaled air are elevated in asthmatic patients. It remains unclear, however, whether the effects of NO in asthma are primarily beneficial or deleterious. NO could perpetuate the inflammatory state through increased plasma leakage from affected postcapillary venules and could damage airway tissue by interacting with superoxide anion to generate toxic peroxynitrite. It has also been reported to suppress the production of the T_{H1} subtype of lymphocytes, thereby favoring enhanced T_{H2} effects. By contrast, the bronchodilatory effects of NO would be desirable in asthma. Moreover, there is growing evidence that NO can be antiviral and can inhibit the production of proinflammatory cytokines, suggesting antiinflammatory effects, particularly during viral exacerbations of asthma.⁸

Catabolic enzymes/inhibitors

Although it can release mediators that we think of as proinflammatory, the epithelium also has intrinsic antiinflammatory, or protective, properties. Cell surface peptidases, most notably neutral metalloendopeptidase 24.11 and aminopeptidase M, reduce the effects of peptide mediators on airway smooth muscle, vasculature, and neural tissue by actively degrading them. Substrates for neutral metalloendopeptidase 24.11 include tachykinins, bradykinin, and enkephalins. Similarly, the epithelium produces a range of protease inhibitors, including secretory leukocyte protease inhibitor, elafin, cystatin C, $\alpha 1$ -antiprotease, and $\alpha 1$ -antichymotrypsin that can prevent tissue damage by enzymes such as elastase, cathepsins B and G, and mast cell chymase.

IMMUNE REGULATION

A relatively unexplored area of epithelial cell biology is the potential role of the epithelium in antigen presen-

tation to lymphocytes. Epithelial cells at all levels of the airways express class II MHC antigens. Normal airway epithelium has relatively low basal levels of HLA-DR on the cell surface, but significantly enhanced expression is seen in patients with asthma or allergic rhinitis. The capacity of epithelial cells to function as HLA-DR-restricted antigen-presenting cells, however, is somewhat controversial. Although one study found no ability to stimulate proliferation of allogeneic T-lymphocytes, another reported that epithelial cells presented antigen to autologous T lymphocytes, although their capacity in this regard was somewhat weak. Interestingly, it has recently been reported that intestinal epithelial cells can present viral antigens to antigen-specific CD8⁺ cytotoxic lymphocytes.⁹ Parallel studies have not yet been performed with infected human respiratory epithelial cells, but if similar data were observed, this could have important implications for our understanding of host responses to respiratory viral infections.

MAINTENANCE OF EPITHELIAL INTEGRITY

Asthma is characterized by epithelial desquamation, although the extent of epithelial damage remains controversial. Although epithelial damage usually occurs at the junction between basal cells and columnar cells, denudation to the level of the basement membrane is also observed. Interestingly, when such damage is induced experimentally, repair begins within an astonishingly short amount of time (20 minutes) as the remaining basal cells flatten and spread to cover the basement membrane.¹⁰ In severely damaged and denuded airways, the basal cells remaining at the site of injury may also secrete products that promote and stimulate repair.¹¹ Epithelial cells produce at least 3 matrix glycoproteins: fibronectin, tenascin, and entactin. Fibronectin is one of the primary matrix components and acts as a substrate for cell adhesion. It also functions as a chemoattractant stimulus for both fibroblasts and epithelial cells, and thus the basal cells remaining in a field of injury may recruit epithelial cells from the margin of the damaged area by secretion of fibronectin. Tenascin is structurally homologous to fibronectin and is increased in the airway tissues of patients with asthma. Entactin is the most recently identified glycoprotein product of the epithelium, and its function is to facilitate basement membrane assembly by binding laminin and type IV collagen.

Several of the epithelial-derived mediators described earlier, including IL-6 and PGE₂, have effects on extracellular matrix deposition, resulting in another level of regulation of repair and remodeling by the epithelium.

CONCLUSIONS

The traditional view of the epithelium as a static barrier is no longer valid because an ever-expanding spectrum of roles for epithelial cells in the inflammatory process has been described. Cytokines, lipid mediators, peptide products, and reactive oxygen species are generated by the epithelium and function to recruit circulating leukocytes to the airway, modulate airway tone, regulate secretions, and

promote antimicrobial and antiviral activity. These effector functions of the epithelium extend to a potentially direct immunoregulatory role and to a contribution to the active process of repair and remodeling of the damaged airway. In asthma and other diseases in which inflammation is central to disease pathogenesis, a better understanding of these functions of the airway epithelium may help direct future therapeutic interventions.

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