

Mechanisms of allergic diseases

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Breakdown in epithelial barrier function in patients with asthma: Identification of novel therapeutic approaches

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Activity Objectives

1. To understand the role of the bronchial epithelium as a chemical, physical, and immunologic barrier to the inhaled environment.
2. To recognize abnormalities in barrier function in patients with asthma.
3. To recognize potential therapeutic approaches to restore barrier function.

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The bronchial epithelium is pivotally involved in the provision of chemical, physical, and immunologic barriers to the inhaled environment. These barriers serve to maintain normal homeostasis, but when compromised, the immunologic barrier becomes activated to protect the internal milieu of the lung. We discuss what is currently understood about abnormalities in these barrier functions in patients with asthma and consider novel therapeutic opportunities that target this key structure. (*J Allergy Clin Immunol* 2009;124:23-34.)

Key words: Asthma, epithelium, tight junction, innate immunity, permeability, allergen, virus

Asthma is an inflammatory disorder of the conducting airways, which undergo distinct structural and functional changes, leading to nonspecific *bronchial hyperresponsiveness* and airflow obstruction that fluctuates over time. It is among the most common

Abbreviations used

BEC: Bronchial epithelial cell
DC: Dendritic cell
EGF: Epidermal growth factor
IP-10: IFN-inducible protein 10
MC: Mast cell
TJ: Tight junction
TLR: Toll-like receptor
ZO: Zonula occludens

chronic conditions in Western countries, affecting 1 in 7 children and 1 in 12 adults. Although at one time considered a single disease entity, asthma subphenotypes are now recognized with differing pathology, clinical expression, responses to treatment, and long-term outcomes.¹ Most asthma exhibits a T_H2-type inflammatory response with upregulation of cytokines of the *IL4* gene cluster linked to *atopy*; however, the indistinguishable pathologic features of nonallergic and allergic asthma and the fact that the great majority of atopic subjects do not have asthma² emphasizes that asthmatic airway inflammation and remodeling can occur independent of atopy.³

We have previously proposed that allergic-type inflammation and aberrant epithelial injury/repair mechanisms were parallel

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Terms in boldface and italics are defined in the glossary on page 24.

phenomena leading to different asthma subtypes⁴ that involved activation of the epithelial-mesenchymal trophic unit, which controls the local tissue microenvironment.⁵ We postulated that in patients with asthma, epithelial susceptibility to damage by environmental agents results in signals that act on the underlying mesenchyme to propagate and amplify inflammatory and remodeling responses in the submucosa. Based on current understanding, many of the persistent inflammatory and structural responses in patients with asthma, including airway allergen sensitization in genetically susceptible individuals, could follow from a defective epithelium, leading to a chronic wound response to repeated environmental injury. Similar mechanisms are now thought to operate in other allergic diseases, leading to enhanced allergen sensitization. Examples

include eczema, in which *polymorphisms* in the *filaggrin* gene affect skin permeability,⁶ and food allergy, in which infection or stress can increase intestinal permeability.⁷

The finding that many novel asthma susceptibility genes identified from hypothesis-independent approaches⁸ are expressed in the epithelium helps place it at the center of asthma pathogenesis. Furthermore, the most frequent environmental risk factors for developing, exacerbating, and prolonging asthma, namely biologically active allergens, air pollutants, environmental tobacco smoke, and respiratory viruses,⁹⁻¹¹ act on the epithelium, thereby strengthening the case for a dynamic interaction between the epithelium and formed elements of the airways in the development of asthma subphenotypes.¹ The purpose of this

GLOSSARY

ANTIOXIDANTS: antioxidants are molecules capable of slowing or preventing the oxidation of other molecules by removing free radical intermediates. They are separated into hydrophilic or hydrophobic antioxidants depending on whether they are soluble in water or in lipids, respectively. Generally, hydrophilic antioxidants react with oxidants in the cell cytosol and the blood plasma, whereas hydrophobic antioxidants protect cell membranes from lipid peroxidation.

ATOPY: Atopy is most commonly defined by increased levels of total and allergen-specific IgE in the serum, leading to positive skin prick test responses to common allergens. Atopy also refers to the genetic tendency for the classic allergic diseases: atopic dermatitis, allergic rhinitis (hay fever), and asthma.

BRONCHIAL HYPERRESPONSIVENESS: Bronchial hyperresponsiveness is also known as airway hyperreactivity. It is an exaggerated constriction of the bronchioles or small airways of the lung in response to physical, chemical, or pharmacologic stimuli. It is typically assessed based on a bronchial challenge test with methacholine or histamine. Bronchial hyperresponsiveness is a hallmark of asthma but also occurs in subjects with chronic obstructive pulmonary disease.

COMPLEMENT: Complement is a biochemical cascade of more than 20 proteins that aid in eliminating pathogens as part of the innate immune response. The complement cascades function to (1) lyse cells, bacteria, and viruses; (2) promote phagocytosis of pathogens by means of opsonization; (3) bind to complement receptors to trigger downstream events; and (4) remove immune complexes. The classical complement pathway, alternative complement pathway, and mannose-binding lectin pathway carry out these functions.

DENDRITIC CELL: DCs are immune cells that take up, process, and present antigen to other immune cells, namely T and B cells in lymphoid tissue. These antigen-presenting cells are primarily found in tissue that serves as an entry point for antigen (ie, skin, lungs, and intestines). DCs are derived from hemopoietic bone marrow progenitors and can be plasmacytoid (pDC) or myeloid (mDC).

FILAGGRIN: Filament aggregating protein (filaggrin) is a key protein that binds to and causes the aggregation of keratins (K1/10). Loss-of-function (null) mutations in the gene encoding filaggrin (*FLG*) result in impaired skin barrier function (ie, ichthyosis vulgaris, atopic dermatitis, or both) and are strongly associated with the development of asthma in patients with atopic dermatitis. R501X and 2284del4 are the most common mutations in the white population, with a prevalence of approximately 7% to 10%.

HAPLOTYPE: A haplotype is a combination of alleles within close proximity on the same chromosome that tend to be inherited together. It can range from as few as 2 loci or as much as an entire chromosome depending on the number of recombination events.

LAMINA PROPRIA: The lamina propria is part of the mucosa, which lines the respiratory tract, the gastrointestinal tract, and the urogenital tract. More specifically, the lamina propria is a thin layer of loose connective tissue that, combined with the epithelium, forms the mucosa.

MAST CELL: MCs reside in various tissues throughout the body and contain histamine and heparin-rich granules. Degranulation can be initiated by means of direct injury, cross-linking of IgE receptors, or activated complement proteins. In addition to allergy and anaphylaxis, MCs exhibit a protective role by being involved in wound healing and defense against pathogens.

MUCIN: Mucins are a class of approximately 19 proteins produced by epithelial tissues. The aminoterminal and carboxyterminal regions of mucin proteins are lightly glycosylated and rich with cysteines to facilitate the formation of disulfide linkages. The central region of mucin proteins is primarily saturated with O-linked oligosaccharides. Increased mucin production is seen in patients with asthma, bronchitis, and chronic obstructive pulmonary disease, with MUC5AC and MUC5B as the most prevalent.

POLYMORPHISM: A polymorphism is also referred to as a single nucleotide polymorphism (SNP) and is a genomic variation occurring when a single nucleotide is different between members of the same species. SNPs can alter genomic DNA-coding sequences, thereby potentially altering amino acid sequences and protein production. In genetic studies SNPs are often assigned an allelic frequency and can be used as biomarkers to determine whether a patient has a genetic predisposition for a particular disease.

REACTIVE OXYGEN SPECIES: Reactive oxygen species are oxygen centered free radicals or reactive non-radical compounds that are generated enzymatically or form as a byproduct of oxygen metabolism and can increase dramatically in times of environmental stress. Reactive oxygen species can induce significant cell damage by damaging DNA, oxidizing fatty acids in lipids, oxidizing amino acids, and inactivating specific enzymes.

TIGHT JUNCTION: A TJ is a closely associated area of 2 cells with membranes that join together, forming a virtually impermeable barrier to fluid. TJs prevent the passage of molecules and ions through the space between cells, thereby forcing them to enter the cells by means of diffusion or active transport to pass through the tissue. Movement of membrane proteins between the apical and basolateral surfaces of the cell is additionally blocked, preserving transcellular transport.

THYMIC STROMAL LYMPHOPOIETIN: TSLP is produced mainly by nonhematopoietic cells as fibroblasts, epithelial cells, and different types of stromal or stromal-like cells. By signaling through the heterodimeric receptor complex of TSLP receptor and IL-7 receptor α , TSLP triggers the release of T cell-attracting chemokines from monocytes and the maturation of DCs. Expression of TSLP is enhanced under atopic conditions, potentiating a T_H2 response.

TOLL-LIKE RECEPTORS: TLRs are a family of membrane glycoproteins that play an important role in the innate immune response by recognizing pathogen-associated molecular patterns, molecules that are shared by pathogens but distinguishable from host molecules. Thirteen TLRs have been identified in human subjects and mice collectively and pair with an adaptor molecule for signaling. TLRs are members of a larger superfamily that includes the IL-1 receptors because of a conserved Toll/IL-1 receptor domain in the cytoplasmic tail.

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review is to consider aspects of the barrier properties of the airway epithelium and to discuss what is currently understood about abnormalities in these barrier functions in patients with asthma, as well as other inflammatory diseases, at the mucosal surfaces. Finally, we will consider novel therapeutic approaches that target this key structure.

THE CHEMICAL BARRIER OF THE AIRWAYS

Each day, the inner surface of the airways is exposed to at least 10,000 L of air, which can contain many particulates, including dusts and microorganisms, as well as noxious gases.¹² These environmental challenges are largely overcome by an efficient filtering system and innate defense mechanisms without the need for an inflammatory response. Within this system, the first line of defense in the lower airways is provided by the bronchial secretions that form both a chemical barrier that detoxifies noxious particles and a physical barrier that traps inhaled particles and, through the action of the mucociliary escalator, continuously transports them from the lower airways to the oropharynx.

Mucus and host defense

Respiratory mucus is a complex secretion, as highlighted by a recent proteomic analysis that identified 191 different proteins in human induced sputum.¹³ The main component of these secretions are the large, highly charged **mucin** molecules (predominantly MUC5B and MUC5AC), which cross-link to form a viscoelastic gel. These multifunctional glycoproteins provide the structural framework of the mucus barrier, prevent dehydration of the epithelial surface, present carbohydrate ligands to sequester pathogens, and can bind to other secreted components, such as host-protective proteins and peptides.¹⁴ Other soluble factors, such as **complement**, immunoglobulins, surfactant proteins, Clara cell proteins, and palate, lung, and nasal epithelium clones (PLUNCs) also contribute to innate defense. PLUNCs are structural homologues of LPS-binding protein and the bacterial permeability-increasing protein, both of which are known mediators of host defense against gram-negative bacteria.¹⁵ Other antimicrobial components include defensins, cathelicidins, lysozyme, lactoferrin, secretory phospholipase A₂, and secretory leukocyte protease inhibitor, which can selectively disrupt bacterial cell walls and membranes, sequester microbial nutrients, or act as decoys for microbial attachment.¹⁶ These host defense molecules can arise from several cellular sources, including airway epithelium, serous gland cells, and myeloid cells, such as neutrophils, macrophages, and lymphoid natural killer cells, or from the circulation. Although β -defensins have antibacterial properties, they are induced in response to viral infection and appear to exert direct antiviral actions against some enveloped viruses and also can impair viral infection.¹⁷ Specific polymorphisms and **haplotypes** in the human β -defensin 1 gene have been associated with asthma and atopy¹⁸; however, no studies have analyzed levels of β -defensin 1 in airways secretions from asthmatic subjects.

Oxidant defense in patients with asthma

The bronchial epithelium is particularly vulnerable to the effects of ambient oxygen, as well as exogenous oxidants from air pollutants (eg, ozone, sulfur dioxide, oxides of nitrogen, and diesel exhaust particles) that are particularly associated with

increased risk of development and exacerbation of asthma. The bronchial secretions contain an array of efficient antioxidant mechanisms for the primary protection of the airways.¹⁹ These include reduced glutathione, antioxidant enzymes, uric acid, vitamins C and E, and proteins such as albumin, transferrin, ceruloplasmin, and lactoferrin, which are capable of binding free iron and other metals that might generate **reactive oxygen species**, as well as the mucins themselves.

In patients with asthma, exposure to air pollutants or endogenously produced reactive oxygen species or reactive nitrogen species appears to lead to oxidative stress, which can result in direct damage to proteins, lipids, and DNA. Many studies suggest that oxidative stress in patients with asthma is linked to increased consumption of **antioxidants** in the airways,¹⁹ as well as polymorphisms in genes encoding glutathione-S-transferase enzymes.²⁰ In a study of children with mild asthma, levels of the enzymes glutathione peroxidase and superoxide dismutase and of the nonenzymatic components of the antioxidant system were all found to be significantly lower in children with asthma compared with those seen in healthy control subjects.²¹ A nitrotyrosine proteome survey in patients with asthma identified an oxidative mechanism of catalase inactivation, and analysis of bronchoalveolar lavage fluid revealed a 50% reduction in catalase activity relative to that seen in healthy control subjects.²² Most recently, children with severe asthma have been shown to have increased biomarkers of oxidant stress in the epithelial lining fluid.²³ Furthermore, there is evidence that the epithelium is deficient in its ability to neutralize oxidant attack through a decrease in antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase, in patients with asthma.^{24,25} Levels of vitamin C and E are also low in patients with mild asthma,²⁶ although longitudinal studies suggest that antioxidant supplementation during adulthood is not an important determinant of asthma, and only vitamin E has a modest protective effect.²⁷

In addition to inhaled and endogenously produced oxidants, epidemiologic studies show that regular exposure to paracetamol (acetaminophen) in the interuterine environment²⁸ in children^{28,29} and in adults³⁰ carries a dose-dependent risk of allergy and asthma. Several biologic mechanisms have been proposed to explain the association between paracetamol consumption and asthma, including glutathione depletion caused by metabolism of the drug. This depletion is proposed to increase the risk of lung tissue damage and ultimately of respiratory disease.³⁰ Other mechanisms that might link paracetamol to the development of asthma include enhanced production of prostaglandin E₂ to promote T_H2 allergic pathways or an IgE-mediated mechanism with acetaminophen as the antigenic agent.³¹ Based on current knowledge, further research into the effects of paracetamol on the incidence of childhood asthma is urgently needed to enable guidelines for the recommended use of paracetamol in childhood to be made.

Our current understanding of the role of oxidative stress in asthma suggests that antioxidant therapy to improve the chemical barrier might be important in optimizing asthma treatment and prevention; however, previous trials with conventional antioxidant therapy in patients with asthma have been largely ineffective. This might be due to the need to select appropriate target populations and strategies that have broad effects on the antioxidant balance.³² However, although restoration of antioxidant defense might be beneficial, this defense system is only one facet of the epithelial barrier that is abnormal in patients with asthma. Thus fixing this aspect alone might not be an effective treatment strategy.

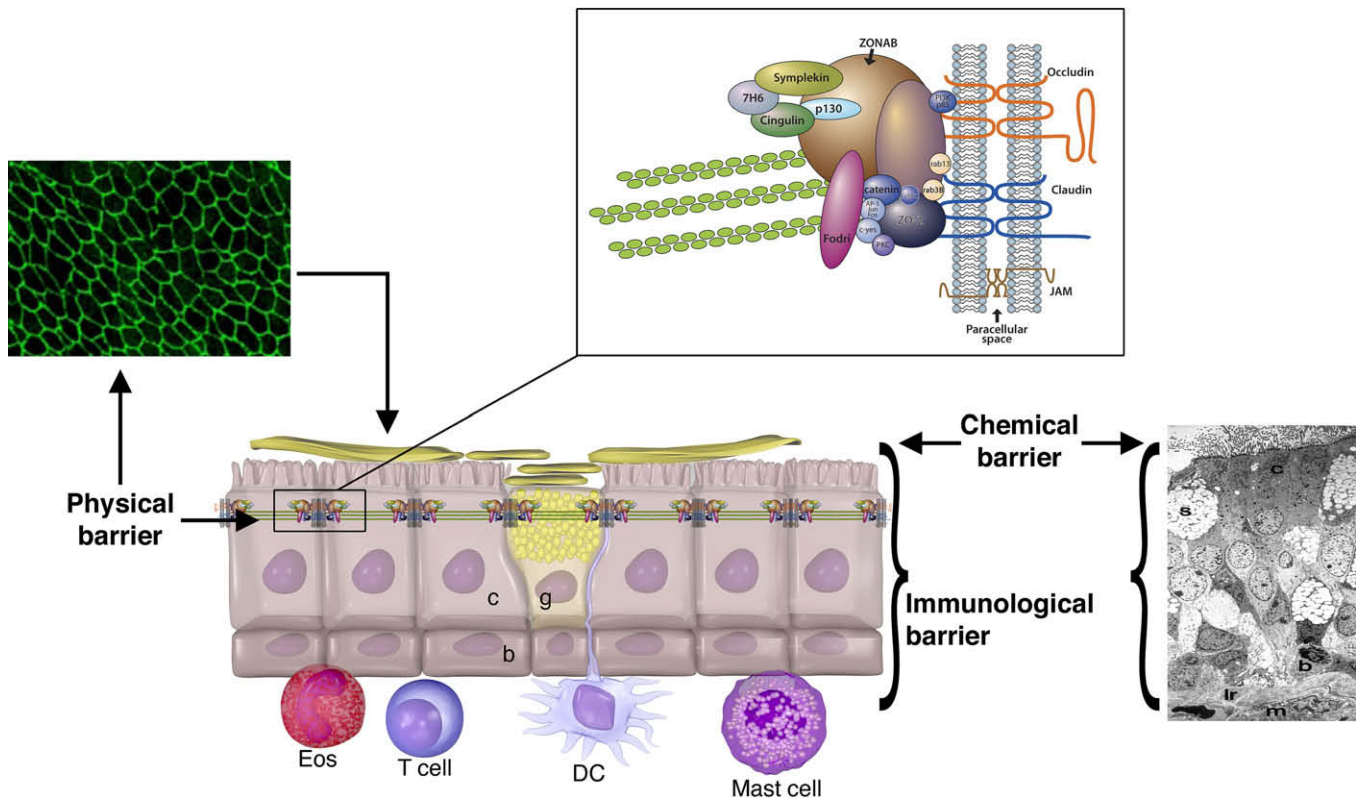


FIG 1. Schematic outline of bronchial epithelium showing basal (*b*), goblet (*g*), and columnar (*c*) cells and its interrelationship with cells of the immune system. Immunofluorescent staining of ZO-1 shows the location of TJs between cells forming the physical barrier (*top left*). The transmission electron micrograph on the right shows bronchial epithelium with goblet cell (*g*), columnar cell (*c*), basal cell (*b*), lamina reticularis (*lr*), and a MC (*m*). Above is a schematic depiction of some of the proteins identified in TJ complexes. Not shown are tricellulin and ZO-3. Tricellulin occurs in tricellular TJs (ie, where 3 epithelial cells meet), and its localization is supported by occludin.³⁷ The ZO proteins bind directly to occludin at the submembranous region of the junction. This interaction is mediated in ZO-1 and ZO-2 by their COOH terminal region, and a similar association is expected for ZO-3.³⁸ 7H6, A 155 kDa phosphoprotein antigen; JAM, junctional Adhesion Molecule; Eos, eosinophil.

THE PHYSICAL BARRIER OF THE AIRWAYS

The mucociliary escalator

The bronchial secretions, with their gel-like properties, play an important role in forming a physical barrier to the environment. The properties of the mucus are dictated in large part by the oligomeric secreted mucins MUC5AC and MUC5B.¹⁴ These multifunctional glycoproteins provide the structural framework of the mucus barrier and serve to trap inhaled particles, which are then moved out of the airways through the action of the mucociliary escalator. The importance of mucins for host defense has been illustrated in models of colitis, in which *Muc2* deficiency leads to inflammation of the colon and contributes to the onset and perpetuation of experimental colitis.³³ Mucins are produced throughout the bronchial tree, particularly by goblet cells and mucus and serous cells of the submucosal glands.¹⁴ In patients with asthma, there is evidence of abnormal mucin production, as demonstrated by an increased number of goblet cells³⁴ and increased amounts of polymeric mucins compared with levels seen in normal induced sputum,³⁵ which might contribute to mucus plugging of the airways. Other factors that contribute to mucus plugging include DNA, serum proteins, and eosinophilic basic proteins. However, in a trial with the mucolytic drug recombinant human deoxyribonuclease as an add-on to standard treatment, there

was no beneficial effect in children presenting to the emergency department with acute asthma exacerbations.³⁶

Epithelial tight junctions

In addition to the secretory mucus, the epithelial cells lining the airway elaborate a selective physical barrier between the external environment and the internal tissues (Fig 1).^{37,38} This physical separation is possible because the cells are structurally and functionally polarized and are linked by macromolecular protein complexes that seal cells together and connect to the actin cytoskeleton to form a coherent layer, which maintains tissue integrity while regulating permeability. Functional patency depends on the coordinate expression and interaction of proteins in cell-cell junctional complexes, including the **tight junction** (TJ), the adherens junction, and the desmosome. The uppermost TJ regulates paracellular permeability and the generation and maintenance of polarity by restricting the movement of proteins in the lipid bilayer.³⁹ The TJ is closely apposed to the E-cadherin-containing adherens junction, with both linking to the actin cytoskeleton. E-cadherin is linked to the actin cytoskeleton through catenin proteins in junctional complexes that host many other interacting signaling proteins and receptors.⁴⁰ Below the adherens junctions are

disc-like desmosomes, which are connected to the keratin filament system and contribute to the cohesive strength of tissues. The adhesive proteins of desmosomes are desmocollins and desmogleins, which interact cytoplasmically with γ -catenin, plakophilin, and desmoplakin to assemble a plaque that interacts with the keratin cytoskeleton in epithelial cells.⁴¹

Proteins of the TJ include the integral membrane proteins of the claudin family, occludin, tricellulin, and junctional adhesion molecule (JAM); the membrane-associated guanylate kinase (MAGUK) family members zonula occludens (ZO) 1, ZO-2, and ZO-3; and the cytoplasmic plaque proteins, cingulin⁴² and 7H6, a 155 kDa phosphoprotein antigen.⁴² The 18- to 25-kDa tetraspan claudin proteins comprise a family of at least 24 proteins whose differential expression and properties determine the permeability properties of epithelia through formation of ion-selective pores.⁴³ The TJ plaque also contains many signaling molecules (Fig 1), including isoforms of protein kinase C, Rho proteins, c-Yes, phosphatidylinositol 3-kinase,^{44,45} and other proteins that have proliferative and differentiating capacity, such as transcription factors, lipid phosphatases, and cell-cycle regulators, with some exhibiting both nuclear and junctional localization.⁴⁶ Thus TJ structures perform more than a physical function; they play key roles in regulating cellular responses, particularly suppression of proliferation to allow epithelial differentiation in a coordinated manner with adherens junctions and extracellular matrix adhesion. Perturbation in the turnover and concentration of junctional proteins is therefore likely to have important implications for the maintenance and stability of the epithelium and the permeability barrier.

TJs are highly regulated by phosphorylation and by their association with the cytoskeleton. Serine and threonine phosphorylation of occludin is abundant in epithelia with intact junctions,⁴⁷ whereas tyrosine phosphorylation of occludin is associated with increased permeability.⁴⁸ In airway epithelial cells inhibiting Rho kinase significantly reduces the smoke-induced permeability to both ions and macromolecules, whereas inhibiting protein tyrosine kinases only reduces smoke-induced macromolecular permeability.⁴⁹ Exposure to either oxidative stress⁵⁰ or acyl-homoserine lactone,⁵¹ a *Pseudomonas aeruginosa* quorum-sensing molecule, also disrupts TJs in Caco-2 colonic epithelial cells through phosphorylation-dependent mechanisms. In the skin house dust mite or cockroach allergens can activate protease-activated receptor 2 to cause opening of TJs and delay epidermal permeability barrier recovery.⁵² Thus it appears that multiple signaling pathways have the potential to affect TJ integrity.

Epithelial barrier abnormalities in patients with asthma

The normal bronchial epithelium consists of a columnar layer of goblet cells and ciliated cells supported by basal cells. The proximal portion of the small distal airways (bronchioles) is also lined by a columnar pseudostratified epithelium, which transitions in more distal airways to a shorter, columnar to cuboidal epithelium in which Clara cells replace mucous secretory cells. In patients with asthma, there is evidence that the bronchial epithelium is damaged, with selective loss of columnar cells⁵³ and enhanced expression of the epidermal growth factor (EGF) receptor (HER1) in areas of damage,⁵⁴ which is consistent with injury having occurred *in vivo* rather than being an artifact caused

during the biopsy procedure. There is also evidence of more subtle damage to the epithelial barrier, with disruption of TJs accompanied by loss of junctional proteins, including ZO-1 and E-cadherin.^{55,56} Although there are no direct data on the phosphorylation status of TJ proteins in patients with asthma, we have previously reported that phosphotyrosine levels are increased in asthmatic epithelium, which would be consistent with reduced barrier function.⁵⁷ Although the pathology of small distal airways has been largely unexamined because of the relative inaccessibility of these structures, there is evidence that there is inflammation, increased mucus plugging, and smooth muscle hyperplasia in the small airways in patients with asthma.⁵⁸ Although a recent proteomics survey of induced sputum reported that levels of Clara cell secretory protein tend to be lower than normal in patients with asthma,⁵⁹ there is no information about whether small airways epithelial barrier properties are different in patients with asthma.

A number of studies have identified agents that have the potential to cause disruption of epithelial TJs in asthma. Endogenously produced cytokines, such as IL-13 and TNF- α , can cause disruption of the epithelial barrier in *in vitro* models,⁶⁰ whereas inhalation exposure to ovalbumin in sensitized mice or rats causes epithelial TJ disruption,^{61,62} which is consistent with inflammation causing barrier disruption. *In vitro* exposure of epithelial cells to environmental factors, such as the dust mite allergen Der p 1,⁶³ fungal allergens,⁶⁴ rhinovirus,⁶⁵ and cigarette smoke,⁴⁹ all cause barrier disruption, measured either as changes in ionic permeability by transepithelial electrical resistance or macromolecular permeability by passage of fluorescein isothiocyanate-labeled dextran. Although environmental agents or proinflammatory cytokines have the potential to cause disruption, it is noteworthy that fully differentiated cultures of epithelial cells from asthmatic donors have evidence of TJ disruption in the absence of exogenous stimuli.⁶⁶ It is currently unclear whether this phenotypic abnormality is due to genetic or epigenetic factors.

Damage to the epithelial barrier in patients with asthma shows similarity to inflammatory conditions of the skin and the gut. By analogy with atopic dermatitis, in which allergic sensitization has been proposed to occur secondary to impairment of skin barrier function,⁶⁷ a similar mechanism might account for penetration and sensitization toward inhaled allergens in patients with asthma. A structurally compromised epithelial barrier might contribute to epithelial fragility in patients with asthma and help explain widespread evidence of stress and increased expression of heat shock proteins⁶⁸ and the cyclin-dependent kinase inhibitor p21^{waf}, which have protective roles in the nonproliferating columnar cells.⁶⁹ Barrier dysfunction might also contribute to abnormal repair responses within the airways, especially subepithelial collagen deposition which might provide an alternative protective barrier in the absence of a functional epithelium and contribute to the characteristic thickening of the lamina reticularis observed in asthma. As will be described below, barrier dysfunction might have implications for the normal function of the immunologic barrier.

THE IMMUNOLOGIC BARRIER OF THE AIRWAYS

The immunologic barrier consists of humoral and cellular components and functions to protect the internal environment of the body by activating or suppressing innate and adaptive immunity. The humoral component is the nonspecific secretion of

immunoglobulins, whereas the cellular component includes macrophages, T cells (both memory and regulatory), **dendritic cells** (DCs), **mast cells** (MCs), B cells, and plasma cells. Epithelial cells contribute to humoral immunity by actively transporting immunoglobulins from the *lamina propria* to the luminal surface, and they contribute to cellular immunity by physically interacting with and secreting cytokines and chemokines that instruct nearby immune cells. Within the airway, homeostasis is maintained by means of active suppression of innate and adaptive immunity through the release of IL-10 and TGF- β by regulatory T cells (CD4⁺CD25⁺ cells) and DCs. Epithelial cells also contribute to this immune suppression through the constitutive secretion of IL-10.⁷⁰

When homeostasis is disrupted by an event such as exposure of the airways to pathogens, the humoral arm of the immunologic barrier forms an extra level of protection in addition to the chemical and physical barriers. IgA, IgG, and IgM, which are exported to the luminal surface of the airways by epithelial cells, coat invading pathogens, which allows for their subsequent removal by means of mucociliary transport or recognition and killing by Fc receptor-expressing cells. If these barriers are overwhelmed, specific activation of the cellular immunologic barrier occurs, resulting in induction of the innate immune response and subsequent activation of adaptive immunity.

Epithelial innate immune responses

As a resident structural cell that lies at the interface between the internal and external environment, epithelial cells of the airways play a pivotal role in the induction of innate immunity. Epithelial cells express pattern-recognition receptors, including **Toll-like receptors** (TLRs), which are activated to release an array of cytokines and chemokines on stimulation.⁷¹ Pattern-recognition receptors are activated by pathogen-associated molecular patterns but can also be activated by some damage-associated molecular patterns, such as calprotectin,⁷² heat shock proteins, high-mobility group box 1 protein, uric acid, and ATP.⁷¹ Calprotectin, a heterodimeric protein complex composed of S100A8 and S100A9, is found in the cytosol of neutrophils, monocytes, and keratinocytes and is an important factor for cell migration and wound healing.⁷² However, cell damage or activation of phagocytes triggers their release into the extracellular space, where they have bacteriostatic properties and become danger signals that activate immune cells and vascular endothelium through interaction with TLR4 or receptor for advanced glycation end products, amplifying the host response to pathogen-derived factors, such as LPS. In an analysis of epithelial genes in patients with chronic rhinosinusitis, expression of *S100A8* and *S100A9* mRNA was significantly decreased when compared with that seen in control subjects,⁷³ suggesting this might impair epithelial repair responses in patients with chronic rhinosinusitis. Although intracellular expression of *S100A8* and *S100A9* has not been evaluated in patients with asthma, a proteomic survey of induced sputum has reported higher levels of calgranulin A (S100A8) in patients with asthma,⁵⁹ suggesting that it might trigger an inflammatory response by acting as a damage-associated molecular pattern. As will be described below, TLRs are also exploited by allergens.⁷⁴

Interactions with cells of the immune system

Within the immunologic barrier, epithelial cells can recruit and modulate cells of the immune system and also affect their survival

through adhesive interactions, selective release of cytokines and chemokines, and expression of cell-surface receptors. DCs in the airways reside between epithelial cells and extend their projections toward the luminal surface.⁷⁵ In the gut intraepithelial DCs sample bacteria through the epithelial layer by means of upregulation of occludin and ZO-1 in response to LPS, which appears to allow sampling of the external environment without compromising the epithelial barrier.⁷⁶ There is evidence to suggest that a similar process also occurs in the airways because DCs (HLA-DR⁺, CD11c⁺ cells) present in the epithelium of patients with allergic rhinitis express the TJ protein claudin-1, implying that DCs can penetrate epithelial TJs to sample environmental antigens.⁷⁷ The importance of the physical barrier in suppressing inflammation in the gut has been shown in recent studies of transgenic mice in which epithelial TJ dysfunction was induced by means of targeted expression of a constitutively active myosin light chain kinase in intestinal epithelial cells.⁷⁸ In these mice the presence of a dysfunctional epithelial barrier activates mucosal immune responses and accelerates the onset and severity of immune-mediated colitis. By analogy, barrier disruption in patients with asthma might contribute to inappropriate activation of the airway mucosal immune response.

Airway epithelial cells also have the potential to recruit DC precursors (monocytes) to the lung through the release of monocyte chemoattractant protein 1 and 4, both of which are present at higher levels in asthmatic subjects.^{79,80} Furthermore, epithelial cells are capable of supporting monocyte differentiation into DCs which possess higher levels of CD80, CD40, and HLA-DR, have an increased capacity to capture and process antigen, and have a reduced capacity to stimulate T-cell cytokine production (IL-5, IL-9, and IL-13). Recent studies suggest that release of type I IFN by epithelial cells is responsible for the regulation of DC maturation and function, suggesting that in a normal homeostatic environment epithelial cells support and control DC differentiation and function.⁸¹ In an inflammatory environment epithelial cells can be activated by IL-1 β , causing release of IL-15, which promotes monocyte differentiation into DCs that express dendritic cell-specific ICAM3 grabbing nonintegrin (DC-SIGN), CD11c (myeloid DCs), CD123 (plasmacytoid DCs), and CCR6, can capture antigen, and promote T-cell proliferation.⁸² In response to diesel exhaust particles, epithelial cells release GM-CSF to promote maturation of immature DC and T-cell proliferation.⁸³ In addition to the release of mediators involved in the recruitment of DC precursors and the modulation of subsequent DC function, epithelial cells can also recruit mature DCs through the expression of the chemokine CCL20. The receptor for CCL20 is CCR6, which is expressed by DCs and upregulated in response to LPS, bacterial antigens,⁸⁴ and house dust mite allergens.⁸⁵ In the latter case induction of CCL20 secretion is not protease or TLR2/4 dependent but relies on β -glucan moieties within the house dust mite extract.⁸⁵ Hence epithelial cells have the capacity to induce the recruitment and differentiation of monocytes into DCs and to affect their subsequent functional responses, as well as to recruit mature DCs. Through these mechanisms, epithelial cells can control the response of DCs to both pathogenic and nonpathogenic antigens, suggesting a key role of this relationship in the development of allergic diseases.

Epithelial cells also participate in the adaptive immune response to infection through the expression of MHC and costimulatory molecules, such as B7.1 (CD80), B7.2 (CD86), and the B7 homologues B7-H1, B7-H2, B7-H3, and B7-DC.⁸⁶⁻⁸⁸ After exposure to a respiratory virus (rhinovirus and respiratory

syncytial virus), epithelial cells upregulate MHC class I,⁸⁸ B7.1, B7-H1 (PDL-1), and B7-DC (PDL-2).⁸⁸⁻⁹⁰ Through upregulation of MHC class I, epithelial cells can promote their own lysis by viral-specific CD8⁺ cytotoxic T cells and, by increasing the costimulatory molecule B7.1, they can actively engage local naive T cells at the infection site to become T_H1 IFN- γ -producing cells. However, upregulation of the inhibitory costimulatory molecules B7-H1 and B7-DC in epithelial cells is involved in the inhibition of IFN- γ production by T cells,⁸⁷ suggesting that inducible epithelial expression of B7 family members in response to viral infection could influence the adaptive immune response and the cytokine environment after viral infection.

In addition to DCs, epithelial cells also interact with several other cell types, including MCs, eosinophils, neutrophils, and T cells, that are relevant to asthma. MCs lie in close proximity to epithelial cells residing in the lamina propria directly below the basement membrane of the epithelial barrier, where they act as sentinels of the immune system. Human airway and nasal epithelial cells express the prosurvival and chemotactic MC-specific mediator stem cell factor,^{91,92} suggesting that epithelial cells can recruit and support local MC survival. Coculture of MCs with epithelial cells results in proliferation of immature MCs,⁹³ increases survival of mature MCs,⁹¹ and increases adhesion of MCs to the epithelium.⁹⁴ Subsequent MC activation by IgE/antigen in this coculture model is reduced, suggesting that in the normal, noninflamed situation epithelial cells actively suppress MC function.⁹⁵ Furthermore, MC activation, resulting in tryptase secretion, can induce epithelial cells to secrete IL-8 and increase intercellular adhesion molecule 1 expression, demonstrating cross-talk between these 2 cell types.⁹⁶ In patients with asthma, MCs relocate from the lamina propria into the epithelial layer, the airway submucosa, and airway smooth muscle layers. Within the epithelium, they have been shown to be a major source of IL-4, IL-5, IL-13, and TNF- α ,⁹⁷ which can initiate and maintain inflammation in asthma, as well as locally disrupt TJs. Furthermore, tryptase can also contribute to the opening of TJs through activation of protease-activated receptor 2.⁵²

The increased recruitment and retention of eosinophils in the airways is a key feature of asthma, and there is evidence to suggest that the epithelium is a major controller of eosinophilic influx and survival. Epithelial cells generate eotaxin and RANTES, which induce eosinophil chemotaxis,⁹⁸ and eosinophils can transmigrate through polarized epithelial monolayers.⁹⁹ Increased survival of eosinophils after culture with epithelial conditioned media suggests epithelial cells also support eosinophil survival through the release of GM-CSF.^{100,101} Furthermore, eosinophils strongly adhere to epithelial cells and show enhanced degranulation compared with eosinophils activated in the absence of epithelial cells.¹⁰² The adherence of eosinophils to epithelial cells can be upregulated after activation of epithelial cells with TNF- α and IFN- γ through a CD18/intercellular adhesion molecule 1-dependent mechanism.¹⁰³ Epithelial cells are also capable of inducing the chemotaxis and survival of neutrophils through the production of several mediators, including IL-8 and GM-CSF.¹⁰⁴ The enhanced activation and survival of granulocytes by epithelial cells is likely to have a detrimental effect on the epithelial barrier in a process termed "epithelial shedding," and activated eosinophils contribute to this phenomenon.¹⁰⁵ Through the increased activation of eosinophils and neutrophils and the resultant damage to the epithelium, the compromised epithelial barrier might allow for the delivery of antigens/aeroallergens to immune cells that

lie beneath the barrier and subsequent activation of T_H2-mediated inflammation.

Under normal physiologic conditions, T cells of the regulatory subset reside in the airways, where they lie either directly underneath the epithelial layer or within the lamina propria and have an immunomodulatory function. In response to infection, there is rapid recruitment of activated T cells to the airways, which are quickly cleared by means of a tightly controlled mechanism of apoptosis once the infection has been controlled. However, a key feature of asthma is an increase in T cells in the airways, which might be at least in part be due to the production of chemokines and growth factors by epithelial cells that contribute to T-cell recruitment and survival. Thus expression of thymus and activation-regulated chemokine, the chemokine for the CCR4-expressing CD4⁺ T_H2 cells, is increased in epithelial cells of asthmatic subjects compared with those of nonasthmatic control subjects.¹⁰⁶ Furthermore, 24 hours after allergen provocation of atopic asthmatic subjects, expression of thymus and activation-regulated chemokine, as well as another CCR4 ligand, monocyte-derived chemokine, is strongly upregulated on airway epithelial cells and is associated with recruitment of CCR4- and CCR8-expressing T cells.¹⁰⁷ Evidence that asthmatic epithelium can also increase T-cell survival has come from tissue-engineering studies using epithelial cells and fibroblasts from healthy or asthmatic donors in a model of the bronchial mucosa. In this model TGF- β released by structural cells from asthmatic subjects decreased the ability of T cells to undergo apoptosis, whereas structural cells from healthy subjects had no effect.¹⁰⁸ Interactions between epithelial cells and T cells are also evident when cytokine release is examined. For example, T_H17 cells produce more IL-17A after coculture with epithelial cells.¹⁰⁹ However, in this model T cells could also induce the release of cytokines from the epithelial cells, with T_H0, T_H1, and T_H2 cells promoting the release of moderate amounts of IL-8, IL-1 β , and GM-CSF, whereas T_H17 cells potentially induced the release of these cytokines. Together, these data suggest a positive feedback loop between T cells and epithelial cells in which each influences the function of the other.¹⁰⁹

Responses to viral infection in asthma

Respiratory viruses, especially rhinoviruses, and allergens are 2 key triggers of asthma exacerbations in children and adults that deserve special mention because they significantly affect the epithelium.^{56,110} Although rhinoviruses usually infect the upper airway epithelium, evidence suggests that they also infect the lower airway epithelium in patients with asthma. Infection can trigger disruption of TJs⁶⁵ and release of proinflammatory cytokines, such as IL-8, eotaxin, RANTES, IFN-inducible protein 10 (IP-10), and macrophage inflammatory protein 1 α .¹¹¹ Our studies comparing responses of primary bronchial epithelial cells (BECs) with rhinovirus infection *in vitro* indicate that some cytokines, such as IL-8 or RANTES, are more sensitive to inhibition by corticosteroids than others, such as IP-10 and TNF- α ; this difference in sensitivity might explain why increased serum levels of IP-10 are a good biomarker of viral infection during acute asthma exacerbations.¹¹¹ Infection also induces expression of the innate antiviral proteins IFN- β and IFN- λ .^{112,113} Our *in vitro* studies have further shown that BECs from asthmatic subjects have reduced type I and III IFN responses and, as a consequence, are more permissive for viral replication.^{112,113} These studies help to explain the tendency of asthmatic subjects to have lingering lower respiratory tract problems as a consequence of rhinovirus

infection and suggest that strategies to boost endogenous IFN levels might help to limit the severity of asthma exacerbations. Further studies are now needed to explore this possibility in virus-induced asthma exacerbations *in vivo*. In support of such an approach, low-dose IFN- α treatment has been tested in patients with severe corticosteroid-resistant asthma, with marked beneficial effect.¹¹⁴

Respiratory viral infection and other proinflammatory stimuli lead to induction of the IL-7–like cytokine *thymic stromal lymphopoietin* (TSLP) in epithelial cells.^{115,116} This cytokine promotes DC survival and activation by means of upregulation of MHC class II and costimulatory molecules to induce the proliferation of naive T cells to T_H2 cells producing IL-4, IL-5, IL-13, and TNF- α .¹¹⁷ Furthermore, TSLP also upregulates OX40 ligand on DCs, which induces activated CD4⁺ T-cell proliferation and expansion of T_H2 memory cells through OX40 ligation on T cells.¹¹⁸ TSLP release by epithelial cells also causes activation of MCs, leading to the release of T_H2 cytokines, which can activate T cells in the absence of DCs.¹¹⁵ Therefore through the release of TSLP, epithelial cells can potently activate 2 sentinels of the immune system that can induce a T_H2 inflammatory response that can be both T-cell dependent (DC mediated) and T-cell independent (MC mediated).¹¹⁹ TSLP levels are increased in patients with atopic dermatitis¹¹⁷ and asthma,¹²⁰ and in our own unpublished *in vitro* studies, we have found that in response to TLR3 agonists, BECs from asthmatic subjects make less IFN- β and more TSLP than those from healthy donors. This bias might influence the subsequent response to viral infection in asthma toward a T_H2 response.

Responses to allergens

Enzymatically active allergens have been proposed to affect epithelial function through a variety of mechanisms, including barrier disruption, activation of protease-activated receptors, and induction of cytokine release. However, the reason why most aeroallergens tend to generate maladaptive effector T-cell responses has been unclear. Most recently, it has been shown that the main house dust mite allergen Der p 2 has structural homology with MD-2, the LPS-binding component of the TLR4 signaling complex. This functional mimicry facilitates signaling through direct interactions with the TLR4 complex, even in the absence of MD-2, and can facilitate activation by LPS.⁷⁴ Thus it has been proposed that Der p 2 (and other lipid-binding allergen proteins) might have adjuvant properties leading to induction of adaptive immunity so that, under conditions of low LPS exposure, they can shift immune responses toward a T_H2 type. Furthermore, in a murine model TLR4 expression on lung structural cells, but not on DCs, was necessary and sufficient for DC activation in the lung and for priming of effector T_H responses to house dust mite. In this study TLR4 triggering on structural cells caused production of the innate proallergic cytokines TSLP, GM-CSF, IL-25, and IL-33.¹²¹ This study emphasizes the importance of the innate immune function of airway epithelial cells in driving allergic inflammation through activation of mucosal DCs.

THERAPEUTIC APPROACHES

Given the central role of the epithelium in controlling tissue homeostasis, it is attractive to consider therapeutic targets and approaches that help restore normal epithelial function in patients

with asthma because these targets might lie upstream of, and control, many aspects of the inflammatory response. When the integrity of the TJ system is compromised, a deleterious immune response to environmental antigens might be elicited. Penetration of antigens activates DCs and innate defenses, leading to increased expression of chemokines and cytokines, recruitment of effector cells, and further loss of barrier function, resulting in breakdown of barrier homeostasis. Thus therapies aimed at controlling the barrier *per se* could potentially alter the outcome in many diseases.

Current treatments

Of the current mainstay treatments for asthma, β_2 -adrenoceptor agonists have no effect on epithelial repair,¹²² whereas the effects of glucocorticoids are controversial. By using a repeat wound model of BECs, chronic treatment with dexamethasone has been reported to extend the lifespan of repeatedly wounded differentiated cultures,¹²² whereas others have reported that corticosteroids induce epithelial apoptosis¹²³ and impair epithelial cell migration.¹²⁴ Although the effect of corticosteroids on bronchial epithelial TJs has not been studied, in cells of the intestine, glucocorticoid treatment has been shown to induce retightening of the intestinal TJ defect in patients with Crohn disease. *In vitro* studies have linked this effect to the ability of the steroid to reduce epithelial permeability by suppression of the TNF- α –induced increase in the myosin light chain kinase gene and protein expression that controls opening of the intestinal TJ barrier.¹²⁵ However, there was no effect on basal barrier function, which might limit the utility of corticosteroids in the restoration of epithelial barrier function in patients with asthma. Of the novel anticytokine treatments that are being developed for asthma, it will be interesting to determine whether either anti-TNF- α or anti-IL-13 have any beneficial effect on airway epithelial barrier function because both of these cytokines increase epithelial permeability *in vitro*.⁶⁰

Growth factors

The ability of growth factors, such as EGF and keratinocyte growth factor, to restore barrier function after injury has been demonstrated in a number of *in vitro* and *in vivo* models.^{62,126} Defects in epithelial barrier function have been observed in patients with Crohn disease and ulcerative colitis (inflammatory bowel disease).^{127,128} The potential of EGF as a novel treatment for inflammatory bowel disease has been demonstrated in a small study involving 12 patients with left-sided ulcerative colitis who were given EGF as an enema daily for 14 days.¹²⁹ This treatment led to disease remission and improvement in disease-activity scores, sigmoidoscopic scores, and histologic scores, which were all significantly better in the EGF group than in the placebo group. Most significantly, this benefit was maintained up to 12 weeks after treatment, suggesting that restoration of the epithelial barrier was able to normalize tissue responses and downregulate inflammation. Although this study was criticized because of a perceived risk that EGF might contribute to malignant transformation by promoting a hyperproliferative phenotype,¹³⁰ it is well established that EGF is present normally in many body fluids (including saliva, gastric secretions, tears, breast milk, and urine),^{131,132} where it plays a role in maintenance of the epithelial barrier and in healing of damaged mucosa. Because EGF receptors are restricted to the basolateral surfaces in noninjured airway epithelia,¹³³ this ensures that receptor and ligand are spatially

separated to prevent inappropriate activation. On epithelial damage, the ligand gains access to the receptor to induce a rapid repair response that is independent of influx of inflammatory cells or activation of local tissue resident cells. A number of studies have identified deficiencies in EGF production in diseases with persistent mucosal damage. These include reduced levels of salivary EGF in diabetic patients linked to the development of oral and systemic complications of diabetes,¹³⁴ reduced gastric and salivary EGF linked to delayed ulcer healing caused by cigarette smoke,¹³⁵ and diminished levels of salivary and serum EGF in premature infants with necrotizing enterocolitis.¹³⁶ Other mitogenic proteins, including antrum mucosal protein 18,¹³⁷ that are present in gut luminal secretions have also been shown to cause barrier restitution and protect TJs from the deleterious effects of oxidant injury in colonic epithelial cells.¹³⁸ Taken together, these studies suggest it might be timely to explore approaches that improve epithelial barrier function in patients with asthma because these might reduce epithelial susceptibility to damage and consequent inflammatory and remodeling responses.

In addition to growth factors, several other peptides have been identified that have the ability to restore barrier function. One such peptide is AT-1001, a peptide inhibitor of zonulin. Zonulin was identified as a human intestinal homologue of ZO toxin,¹³⁹ a *Vibrio cholerae* enterotoxin. This single polypeptide chain increases intestinal mucosal permeability by reversibly altering TJ structure.¹⁴⁰ Cloning of zonulin suggests that it belongs to a family of serine proteases with structural similarities to a series of growth factors, including EGF.¹⁴¹ In the intestine it has been demonstrated that in genetically susceptible individuals gluten and its toxic derivative gliadin activate the zonulin pathway in the jejunum, enabling the entry of gliadin to the submucosa¹⁴² and activation of the innate immune response in celiac disease. AT-1001 has been studied in a proof of concept for management of patients with celiac disease on dietary gluten and has been found to improve barrier function and reduce proinflammatory cytokine production,¹⁴³ supporting the role of zonulin in disease pathogenesis. It would therefore be of interest to examine zonulin expression in airways and its regulation in patients with asthma.

Antiproteases

It has been proposed that focal degradation of airway TJs by allergen-derived proteases can facilitate access of allergen (both enzymatically active and enzymatically inactive bystander allergens) to the immune system.⁶³ Although it might be argued that the antiproteases contained in the airways lining fluid should inactivate inhaled proteases, the elastase inhibitor elafin is particularly sensitive to the biologic activity of the Der p 1 protease,¹⁴⁴ which might increase the susceptibility of patients with allergic diseases to infection. Consequently, inhibition of allergen protease activity is being considered for therapeutic intervention or prevention of allergic asthma and rhinitis.¹⁴⁵ Although feasible, this type of approach needs to be able to inactivate a broad range of allergenic proteases and will have to be maintained at levels sufficient to inhibit allergens as they are constantly inhaled.

Asthma susceptibility genes

Recent genetic studies have identified several novel asthma susceptibility genes, including *ADAM33*, *PHF11*, *DPP10*, *GPR154*, *ORMDL3*, *CH13L1*, and *CYFIP2*.^{8,146} However, in

most cases the biologic functions of the proteins encoded by these genes and their contribution to the development of asthma are still poorly understood. Some of these genes, including *DPP10*,¹⁴⁷ *GPR154*,¹⁴⁸ and *CH13L1*,¹⁴⁹ and several epithelial differentiation genes⁸ have been localized to the epithelium. For example, dipeptidylpeptidase 10 protein expression is increased in the airways of allergic rats.¹⁵⁰ It belongs to a family of peptidases that cleave terminal dipeptides from cytokines and chemokines, but it has also been described as a general modulator of voltage-gated K(+) channel inactivation in neuronal cells.¹⁵¹ Although this peptidase has been proposed as a potential new target for asthma therapy,¹⁴⁷ further work will be required to understand its role in asthma. *CH13L1* has also been associated with asthma¹⁴⁹ and is expressed in airway epithelium.¹⁵² It binds chitin but has no chitinase activity. However, it is upregulated in inflamed mucosa in a murine model of inflammatory bowel disease and exacerbates intestinal inflammation by enhancing bacterial adhesion and invasion in colonic epithelial cells.¹⁵³ If it serves a similar role in mucosal defense in the airways, inhibition of *CH13L1* activity might be a novel therapeutic approach. Another epithelially expressed asthma susceptibility gene is the previous orphan *GPR154* (also known as *GPRA*),¹⁴⁸ which has been identified as the neuropeptide S receptor.¹⁵⁴ The neuropeptide S–neuropeptide S receptor system regulates important biologic functions, such as sleeping/wakening, locomotion, anxiety, and food intake; however, its role in the asthmatic epithelium remains to be determined. Ultimately, asthma susceptibility genes should represent ideal targets for therapy because they are close to the origins of the disease. However, before this can occur, further work will be required to understand their roles in the airways and their genetic contribution to asthma.

CONCLUDING COMMENTS

In summary, the epithelial barrier is a complex structure that plays a central role in tissue homeostasis. Dysregulation of the barrier occurs in a variety of inflammatory diseases and is particularly associated with orchestration of T_H2-type inflammatory diseases. Further research into this area should focus on the abnormal function of the asthmatic epithelium in the context of the immunologic barrier and its interface with innate and adaptive immune cells, especially in early life during the development of atopic disease.

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