Epithelial barriers in allergy and asthma

Peter W. Hellings, MD, PhD,[a,b,c,d] and Brecht Steelant, PhD[c,e]

Leuven and Ghent, Belgium; Amsterdam, The Netherlands; and Crete, Greece

The respiratory epithelium provides a physical, functional, and immunologic barrier to protect the host from the potential harming effects of inhaled environmental particles and to guarantee maintenance of a healthy state of the host. When compromised, activation of immune/inflammatory responses against exogenous allergens, microbial substances, and pollutants might occur, rendering individuals prone to develop chronic inflammation as seen in allergic rhinitis, chronic rhinosinusitis, and asthma. The airway epithelium in asthma and upper airway diseases is dysfunctional due to disturbed tight junction formation. By putting the epithelial barrier to the forefront of the pathophysiology of airway inflammation, different approaches to diagnose and target epithelial barrier defects are currently being developed. Using single-cell transcriptomics, novel epithelial cell types are being unraveled that might play a role in chronicity of respiratory diseases. We here review and discuss the current understandings of epithelial barrier defects in type 2–driven chronic inflammation of the upper and lower airways, the estimated contribution of these novel identified epithelial cells to disease, and the current clinical challenges in relation to diagnosis and treatment of allergic rhinitis, chronic rhinosinusitis, and asthma. (J Allergy Clin Immunol 2020;145:1499-509.)

Key words: *Epithelial cell, tight junction, airway inflammation, epigenetics*

Abbreviations used

AR:	Allergic rhinitis
CDHR3:	Cadherin-related family member 3
CFTR:	Cystic fibrosis transmembrane conductance regulator
COPD:	Chronic obstructive pulmonary disease
CRS:	Chronic rhinosinusitis
CRSwNP:	Chronic rhinosinusitis with nasal polyps
HDAC:	Histone deacetylase
ORMDL3:	Orosomucoid-like 3
PCDH1:	Protocadherin 1
TJ:	Tight junction(s)
ZO:	Zonula occludens

The epithelium lining the nasal and bronchial mucosa has 3 major functions in maintaining a healthy state of the respiratory mucosa: that is, physical barrier function, innate immune defense function, and mucociliary clearance of inhaled particles.[1] These mechanisms aim to prevent inflammation and disease despite the inhalation of infectious agents, pollutants, or allergens. Most environmental molecules are trapped in the mucus layer of the host mucosa and are cleared from the surface by the continuous and coordinated ciliary movements. In addition, epithelial cells produce different cytokines and chemokines that attract and activate inflammatory cells that aid in removing or neutralizing foreign molecules.[2] However, excessive activation of epithelial cells might initiate the onset of diseases such as asthma, allergic rhinitis (AR), and chronic rhinosinusitis (CRS). Another mechanism by which normal epithelial cell function is hampered is through the disruption of the epithelial barrier function. Allergens, pathogens, and pollutants have been shown to cleave ***tight junctions*** (TJs) between epithelial cells,[3] thus facilitating their access to immune cells residing in the vicinity of epithelial cells. We found more and higher migration of bronchially applied ovalbumin from the airway lumen to the airway vessels in allergic versus control mice.[4] Airway diseases such as AR, CRS, asthma, or chronic obstructive airway disease (COPD) are characterized by epithelial barrier dysfunction, including TJ defects and increased epithelial permeability.[5-8] The purpose of this review was to provide an overview of the complexity of the airway epithelium, to discuss the role of epithelial dysfunction in airway diseases, as well as the consequences of increased permeability on the onset and chronicity of disease. In addition, we will discuss the mechanisms contributing to increased permeability and how this can be measured and reversed as a potential novel therapeutic target.

CELLULAR DIVERSITY IN THE AIRWAY EPITHELIUM

The airway epithelium is a dynamic tissue that undergoes continuous but slow renewal to maintain a pseudostratified structure.[9] Considering that the airways are repeatedly exposed to

From [a]the Clinical Department of Otorhinolaryngology, Head and Neck Surgery, University Hospitals Leuven, Leuven; [b]the Department of Otorhinolaryngology, Academic Medical Center, University of Amsterdam, Amsterdam; [c]the KU Leuven Department of Microbiology, Immunology and Transplantation, Allergy and Clinical Immunology Research Unit, Leuven; [d]the Department of Otorhinolaryngology, University Hospital Ghent, Laboratory of Upper Airway Research, Ghent; and [e]the Department of Otorhinolaryngology, Head and Neck Surgery, University of Crete School of Medicine, Heraklion, Crete.

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Corresponding author: Peter W. Hellings, MD, PhD, Clinical Division of Otorhinolaryngology, Head and Neck Surgery, University Hospitals Leuven, Herestraat 49, 3000 Leuven, Belgium. E-mail: Peter.hellings@kuleuven.be.

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Terms in boldface and italics are detailed in the glossary on page 1500.

environmental molecules, maintaining airway homeostasis must be tightly controlled. Based on structural, functional, and biochemical properties, the human airway epithelium consists of the predominant ciliated epithelial cells, mucous-secreting goblet cells, club cells, and airway basal cells[10] (Fig 1). In chronic airway diseases, however, the constant renewal of the epithelium can lead to a disbalance in epithelial cell types, altered epithelial cell activation, or increased permeability, and thus impair normal airway epithelial function.[11]

Common airway epithelial cells

Ciliated epithelial cells are the major cell type within the airways. These cells are terminally differentiated and originate from club cells and/or airway basal cells.[10] The conversion from airway basal cells to ciliated epithelial cells is tightly regulated by the conserved Notch signaling pathway. Suppression of Notch signaling promotes ciliated cell fate, whereas high levels of Notch promote the differentiation toward mucus-secreting goblet cells.[12,13] Type 2 cytokines such as IL-4 and IL-13 stimulate Notch signaling,[14,15] which results in increased number of mucus-secreting goblet cells as found in asthma, CRS, and other diseases.[16] Ciliated epithelial cells contain a large number of cilia, which are necessary for the mucociliary clearance. Reduced ciliary beat frequency, shortened cilia, or ciliary depletion are features of the airway epithelium of patients with asthma and patients with AR.[17]

Mucus-secreting goblet cells are secretory cells that contain vesicles with tightly packed mucin granules and surfactant proteins.[18] The primary function of these cells is to secrete mucins onto the internal surface of the airways so that environmental molecules can be trapped. Muc5AC and Muc5B are considered the major mucin proteins in the airways.[19] In health, there is a fine equilibrium between the production of these mucins and its clearance. Excessive goblet cell differentiation, driven by IL-4 and IL-13, disturbs the balance of Muc5AC and Muc5B, a phenomenon associated with asthma, AR, and CRS. Recently, 3 transcriptionally distinct subtypes of goblet cell were defined, that is, immature goblet cells, goblet-1 cells, and goblet-2 cells.[20] Goblet-1 cells are characterized by genes encoding for key mucosal proteins Trefoil factor-1 and -2, and Muc5B, and secretory regulators such as Lman1l. Goblet-2 cells specifically secrete Demilune cell and parotid proteins 1-3, which is a lectin-like secreted protein that aggregates bacteria, and Lipf, a secreted gastric lipase that hydrolyzes triglycerides. The function of these subtypes during health and disease, however, is currently not known.

Airway basal cells are stem-cell–like progenitor cells found in upper and lower airways, that is, depending on proper regulation of Notch signaling, give rise to ciliated, mucus-secreting goblet cells, or other specialized epithelial cells.[21] Basal cells are firmly attached to neighboring epithelial cells and anchor the epithelium to the basal membrane via ***hemidesmosomes***.[22] They are relatively undifferentiated and characteristically express high levels of cytokeratin 5 and cytokeratin 14, and the transcription factor transformation-related protein 63.[23] At steady state, basal cells are rather quiescent. However, in response to injury, basal cells are rapidly activated and are temporally detached from the basal membrane to migrate toward the epithelial culprit for the formation of a provisional barrier.[24] Aside from their role in tissue maintenance, basal cells are capable of mediating innate immune responses. Amatngalim et al reported that basal cells produce RNase7, an antimicrobial protein, in response to cigarette-induced epithelial injury.[25] Other innate immune mediators, including β-defensin-2, lipocalin 2, IL-6, IL-8, and CCL20, were also upregulated in basal cells. In patients with COPD, a subset of IL-33–secreting basal cells has been reported, which was associated with an IL-13 and mucin gene signature.[26] Recently, transcriptionally distinct basal cell subsets, which expressed *IL-33,* thymic stromal lymphopoietin (*TSLP*), or an *IL-4/IL-13* gene signature, were reported in bronchial biopsies of patients with asthma[27] and in nasal polyps[28] from patients with CRS with nasal polyps (CRSwNP). The observation that airway basal cells contribute to innate immune mechanisms is novel and emphasizes a crucial role for these cells in host-environment interactions and epithelial repair in pathology versus health.

Finally, *club cells* are secretory cells of the small airways and differentiate from basal cells in a Notch-dependent manner. These cells are dome-shaped cubical nonciliated cells that secrete a specific protein belonging to the secretoglobin family (SCGB1A1).[29]

GLOSSARY

ADHERENCE JUNCTIONS: Junctional complexes whose cytoplasmic face is linked to the actin cytoskeleton and are involved in maintaining epithelial cell-to-cell adhesion.

CONFOCAL LASER ENDOMICROSCOPY: An endoscopic procedure developed to image the mucosal layer of the gastrointestinal tract with very high magnification and resolution based on either tissue reflectance or fluorescence.

CRISPR-CAS9: A novel genetic engineering technique based on the bacterial CRISPR-Cas9 antiviral defense system that allows for targeted modification of the genomes of living organisms.

DESMOSOMES: Junctional complexes that are localized spot-like adhesions arranged on the lateral sides of epithelial plasma membranes and attach to adjacent cells to help maintain cell-to-cell adhesion.

ENDOTYPE: A subtype of a disease condition that is characterized by a distinct pathophysiologic mechanism.

EPIGENETIC REGULATION: Regulation of gene expression in a way that does not entail a change in the primary DNA sequence.

GENOME-WIDE ASSOCIATION STUDIES: Observational studies of the genomes of a large number of individuals to determine which genetic variants are associated with a particular trait.

HEMIDESMOSOMES: Junctional complexes with a similar structure to desmosomes but connect basal epithelial cells to the basement membrane.

IONOCYTES: A recently discovered lung cell type that is characterized by high expression of the transcription factor Foxi1, the vacuolar-type H^+-ATPase proton pump, and cystic fibrosis transmembrane conductance regulator.

NEUROENDOCRINE CELLS: Cells that release hormones into the blood in response to neurotransmitters released by nerves or neurosecretory cells.

SINGLE-CELL RNA SEQUENCING: A cutting-edge genomic approach to generate gene expression profiles of individual cells.

SOLITARY CHEMOSENSORY CELLS: A rare epithelial cell population that contains apical microvilli and use their sensory capabilities to detect pathogens and allergens attempting to infiltrate the epithelium with resultant activation of type 2 immunity. Examples include brush cells in the lungs and tuft cells in the intestines.

TIGHT JUNCTIONS: Extracellular junctional complexes, consisting of both transmembrane and cytoplasmic proteins, that prevent paracellular leakage of solutes and water through the epithelium.

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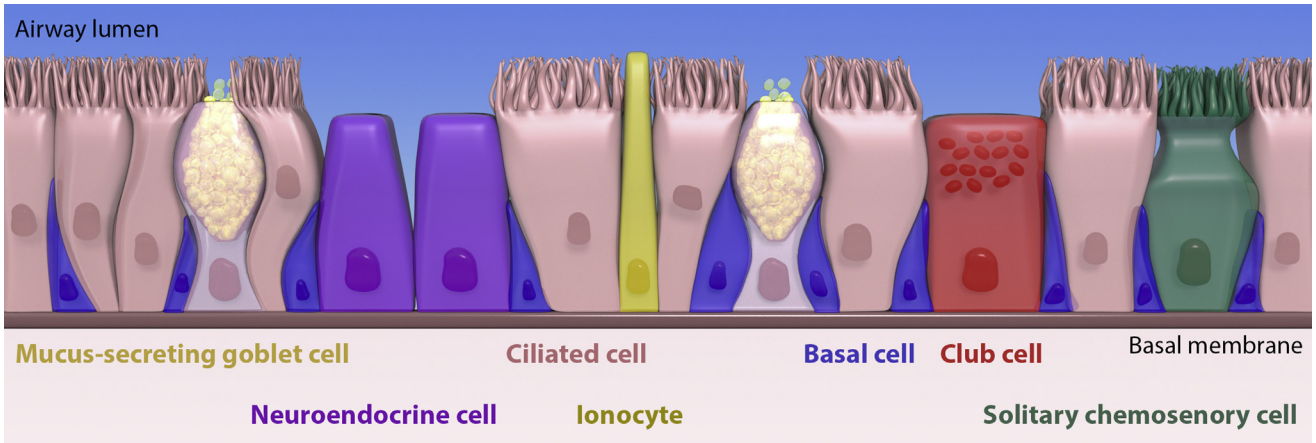

FIG 1. Common and rare cell types of the human airway epithelium. Ciliated epithelial cells, airway basal cells, and club and mucus-secreting goblet cells are regarded as common epithelial cell types. Mucus-secreting goblet cells and club cells secrete mucins and other bioactive compounds that form together with cilia the mucociliary clearance. Neuroendocrine cells, ionocytes, and solitary chemosensory cells are rare, but specialized cells in the airways.

In response to epithelial injury, club cells have the ability to differentiate into ciliated and mucus-secreting goblet cells, a process driven by the intercellular junctional protein E-cadherin.[30] Reduced club cell numbers and SCGB1A1 are reported in patients with COPD, and in patients with asthma and was associated with disease severity.[29,31] Recently, with the use of ***single-cell RNA sequencing***, Montoro et al[20] described a novel subset of club cells in murine airways. Hillock club cells express squamous epithelial markers *Krt4* and *Krt13*, are only found in epithelial regions with high turnover, and have characteristic barrier function (claudin 3) and immunomodulatory (galectin 3 and annexin A1) properties. Whether hillock club cells occur in human airways is currently not known.

Novel, rare airway epithelial cells

More recently, single-cell RNA sequencing allowed the characterization of 3 additional, specialized but rare cell types in the human and mouse airway epithelium, that is, ***neuroendocrine cells***, ***solitary chemosensory cells***, and ***ionocytes***.[20,32]

Neuroendocrine cells occur either as isolated cells or are organized in small clusters called neuroendocrine bodies. Neuroendocrine cells are strategically positioned at airway branch points where allergens and other harmful substances accumulate.[33] These cells contain dense granules containing a wide variety of neuropeptides, amines, and neurotransmitters and are innervated by the sympathetic and parasympathetic nervous system.[34] Historically, neuroendocrine cells were thought to play a fundamental role during early lung development.[35] Neuroendocrine cells serve as airway chemoreceptors that monitor airway status and signal this to other lung cells or to the brain through synapses with the nervous system.[36] More recently, Sui et al[37] provided mechanistic evidence that neuroendocrine cells are critical orchestrators of asthma responses via the activation of group 2 innate lymphoid cells and mucus-secreting goblet cells. Neuroendocrine cells release calcitonin gene–related peptide, which activates group 2 innate lymphoid cells that further promote T_H2 allergic responses. Release of γ-aminobutyric acid was shown to control goblet cell hyperplasia without altering other immune cell function. Increased numbers of neuroendocrine cells have been reported in various lung diseases, including asthma,[38] COPD,[39] and small lung carcinoma.[40] No data on the presence of neuroendocrine cells are currently available for the upper airways.

Solitary chemosensory cells, often called tuft cells due to the presence of an apical microvilli tuft, are peculiar cells in the airway epithelium. The function and signaling pathways of solitary chemosensory cells are quite similar to those of intestinal tuft cells, that is, regulating type 2 immunity.[41] Solitary chemosensory cells express transient receptor potential cation channel subfamily M member 5, the sweet and bitter taste receptor family of G protein–coupled receptors, and succinate receptor-1 that signal through their downstream intracellular effectors, including alpha-gustducin and phospholipase Cβ2.[42-45] Although solitary chemosensory cells were already discovered in the lower airways in the 1970s by electron microscopy,[46] the physiology of these cells is only very recently unraveled. In mice, activation of bitter taste receptors triggers the activation of trigeminal afferent nerves and cause neurogenic inflammation. In the human nose and sinuses, bitter agonists stimulate solitary chemosensory cells that triggers the release of prestored antimicrobial peptides, including β-defensins 1 and 2, from surrounding ciliated and mucus-secreting goblet cells.[42] Defensins are antimicrobial peptides that permeabilize bacteria and kill fungi, emphasizing a crucial role for solitary chemosensory cells in immunity against various pathogens. Furthermore, these cells serve as a unique reservoir of epithelial IL-25.[47] IL-25 is an early epithelial-derived cytokine involved in the type 2 inflammatory responses often seen in allergic asthma and CRSwNP.[48] Solitary chemosensory cells are enriched in the inflamed tissue from patients with allergic fungal rhinosinusitis, and the proliferation and/or differentiation of these cells appears to be stimulated by fungal extract exposure *in vitro*.[49]

Ionocytes were recently identified as a novel cell type in mouse and human airway epithelium,[20,50] and stem from basal cells under the control of Notch signaling. These cells account for only 1% of murine airway epithelial cells and reside at multiple levels of the airway tree. Ionocytes highly express the cystic fibrosis transmembrane conductance regulator (CFTR). More specifically, more than 50% of all detected *Cftr* transcripts are expressed by ionocytes, whereas the common ciliated epithelial cells only express 1.5% of total *Cftr* transcripts.[20] A similar observation was found for human pulmonary ionocytes.[50] Interestingly, CFTR

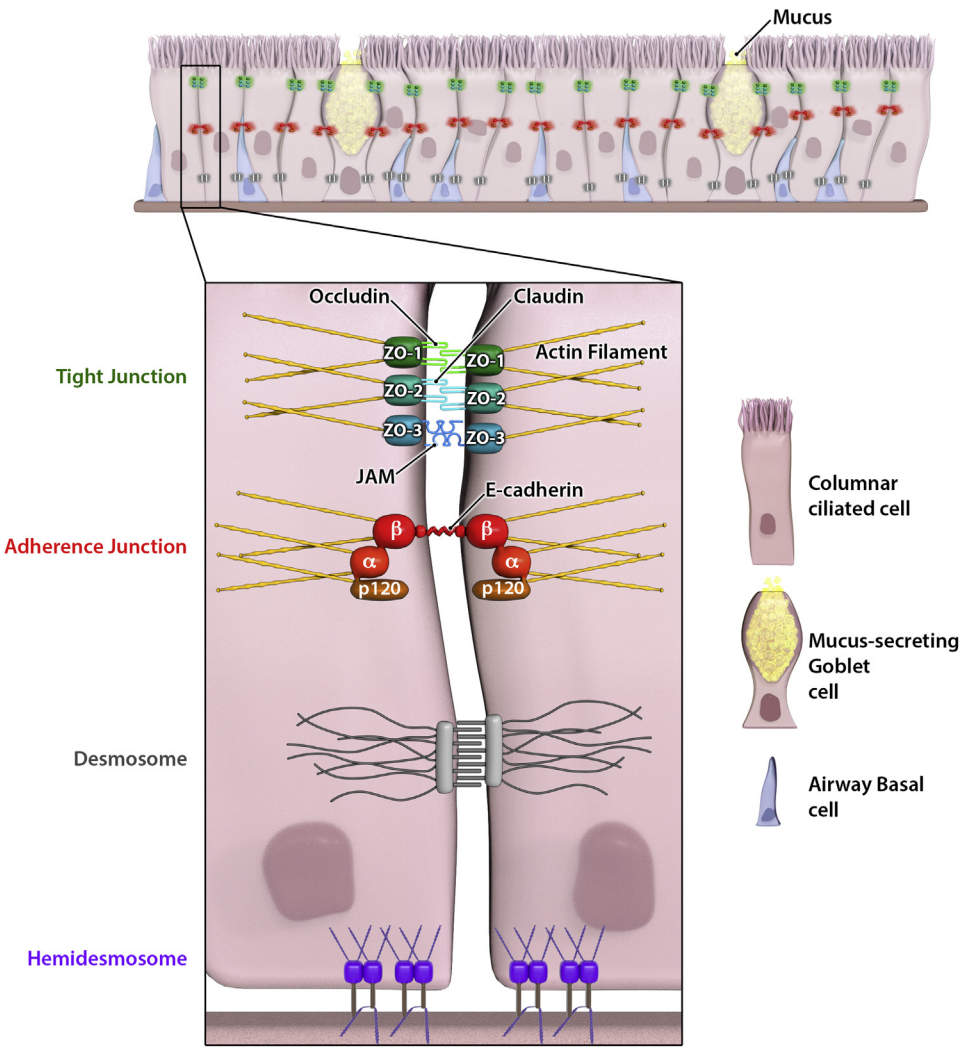

FIG 2. Schematic illustration of different intercellular junctions in the airway epithelium. TJs, adherence junctions, and desmosomes mediate intercellular adhesion and regulate transport of macromolecules, ions, and water. TJs consist of transmembrane proteins: occludin, claudins, and the IgG-like family of JAMs that are linked to cytoplasmic adapter proteins such as ZO-1, ZO-2, and ZO-3. Adherence junctions are located below TJs and are composed of E-cadherin-catenin complexes. Hemidesmosomes ensure the attachment of basal cells and the other epithelial cells to the basal membrane. α, Alpha-catenin; β, beta-catenin; *JAM*, junctional adhesion molecule.

contains a C-terminal interaction domain that regulate TJ assembly and epithelial differentiation.[51] Ruan et al[51] found that CFTR inhibition reduced zonula occludens (ZO)-1 expression and decreased epithelial cell differentiation, which progressively weakened epithelial tissues. Because ionocytes are the major source of CFTR, these cells might potentially play a crucial role in regulating epithelial barrier function. Furthermore, ionocytes are coenriched for the proton-secreting V-ATPases, suggesting a role in regulating luminal pH and mucus viscosity that could be relevant for the pathology of cystic fibrosis.[52] Studies on single-cell expression patterns in airway epithelial cells from patients with cystic fibrosis will help us to better understand the cellular basis of cystic fibrosis and the role of ionocytes in this process.

MAINTAINING EPITHELIAL BARRIER FUNCTION

Chemical and physical barrier

To minimize the contact with the airway epithelium, most environmental molecules are trapped in the mucus layer and cleared from the airways by ciliary movements. The production of airway mucus is a tightly regulated process of major importance to maintaining effective epithelial barrier function. As stated earlier, the balance between Muc5AC and Muc5B is essential for healthy mucus. An increase in Muc5AC over Muc5B increases the viscosity, decreasing ciliary beating and increasing the likelihood of environmental molecules hitting airway epithelial cells. In asthma, CRS, AR, or cystic fibrosis, dysregulated mucin production is reported.[53,54]

The mucociliary layer, however, does not establish a substantial barrier to the external environment. The effectiveness of the physical barrier arises from the coordinated interaction between neighboring epithelial cells via cell-cell adhesion complexes (Fig 2).[11] TJs are the most apically located intercellular junctions and are key regulators of paracellular permeability. Depending on the distribution of specific molecules, TJs provide epithelia with a size- and ion-selective permeability and limit the transport of macromolecules.[11,55,56] TJs consist of transmembrane proteins of the claudin family, occludin, tricellulin, and junctional adhesion

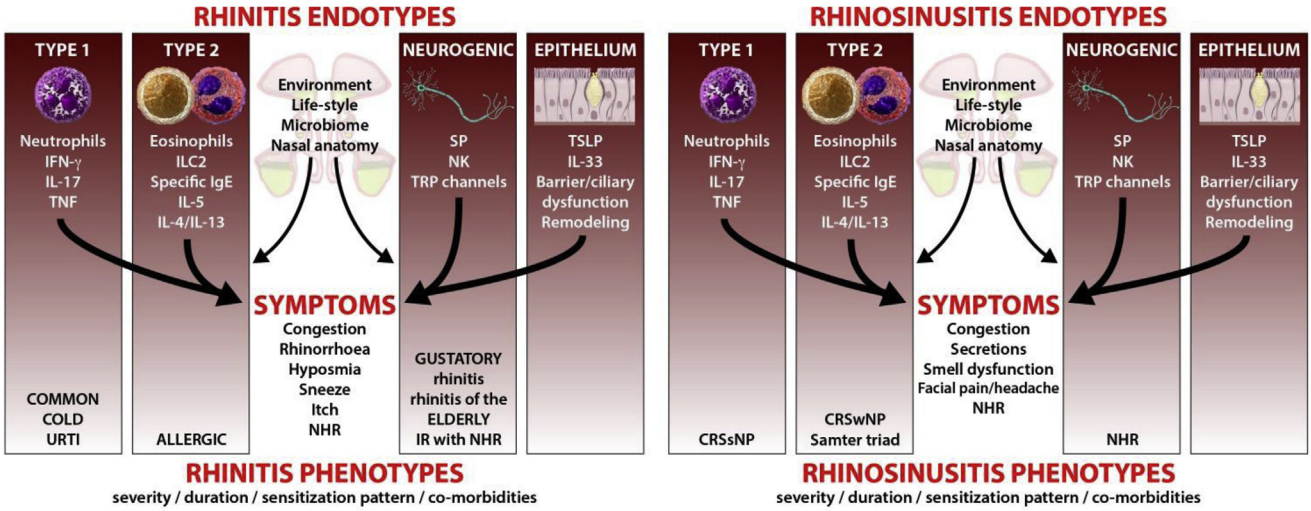

FIG 3. Endotypes of rhinitis and CRS, as currently understood with epithelial barrier endotype as entity. All patients with chronic rhinitis and CRS present with a clinical phenotype that has 1 or more inflammatory endotypes. For the sake of ease of comprehension, endotypes are presented as 4 major separate entities underlying chronic rhinitis and CRS, with the epithelial endotype being only recently recognized. In real life, most patients present with a so-called mixed endotype underlying the clinical presentation. *CRSsNP*; CRS without nasal polyps; *IR*, idiopathic rhinitis; *NHR*, nasal hyperactivity; *NK*, Neurokinin; *SP*, substance P; *TRP*; transient receptor potential; *URTI*, upper respiratory tract infection.

molecules, whereas ZO-1, ZO-2, and ZO-3 molecules are the major TJ-associated cytoplasmic proteins. ***Adherence junctions*** are located directly below TJs and provide intercellular adhesion to maintain epithelial integrity. Adherence junctions are composed of cadherin-catenin complexes and perform multiple functions including initiation and stabilization of TJs, regulation of the actin cytoskeleton, intracellular signaling, and transcriptional regulation.[57] ***Desmosomes*** are located around the midpoint of epithelial cells and provide mechanical stability to the airway epithelium via its strong contact with the intermediate filament cytoskeleton. Finally, hemidesmosomes aid in attaching the epithelial layer to the basal membrane. Together, all these junctional structures do more than just building a physical barrier, as they also regulate epithelial permeability, cell proliferation, and differentiation. Disturbance of this proper regulation will therefore likely have an important implication for the airway epithelium.

Maintaining epithelial barrier function

In response to injury, the airway epithelium rapidly repairs epithelial culprits to protect the body from environmental molecules. Airway basal cells fulfill a pivotal role in this process as they migrate toward damaged regions and completely reconstitute a pseudostratified airway epithelium. Abnormal basal cell proliferation and differentiation has been observed in chronic airway diseases. More specifically, *ex vivo* cultured basal cells of nasal polyps of patients with CRSwNP or basal cells from bronchial biopsies in patients with asthma show reduced capacity to proliferate compared with their respective controls.[58] Building further on this, a recent study by Ordovas-Montanes et al[28] found a major role for type 2 cytokines driving this aberrant basal cell state in CRS. More specifically, they observed an intrinsic IL-4/IL-13 gene signature in basal cells and their progeny that was maintained for several passages *in vitro*. Consequently, the allergic airway epithelium is trapped in an undifferentiated state with reduced cellular and functional diversity. Blocking the IL-4 receptor alpha subunit reduced IL-4/IL-13 signaling pathways in basal cells, emphasizing that basal cell memory can be therapeutically restored.

EPITHELIAL LEAKINESS AND PATHOLOGY

Multiple studies support the concept of the airway epithelial barrier being structurally and functionally disrupted in asthma, CRSwNP, and AR. Disruption of the epithelial barrier in allergic asthma is associated with TJ defects and reduction in adherence junctions and desmosomes.[7,59,60] Of note, increased epithelial permeability was higher in severe compared with mild asthma, without differences in increased permeability in allergic versus nonallergic asthma.[61] We observed decreased expression of occludin and ZO-1 in patients with AR compared with healthy controls, which was associated with disease severity.[5] Increased epithelial permeability, with irregular and decreased expression of the TJ molecules occludin and ZO-1, was found in biopsy specimens from patients with CRSwNP[6] and can be considered as a pivotal player in pathology (Fig 3).[62] Apart from the T_H1, T_H2, and neurogenic endotypes of rhinitis and CRS, an epithelial ***endotype*** can be considered,[63] knowing that mixed endotypes are most prevalent in patients.[64] Type 1 and type 2 endotypes are best characterized and well described since decades. The neurogenic and epithelial endotypes are receiving more attention given the emerging knowledge on mechanisms underlying the contribution of the nervous system to inflammation[65] and the role of epithelium in inflammation.[55] In the context of epithelial dysfunction, it is interesting to note that CRSwNP is characterized by a higher degree of epithelial permeability compared with CRS without nasal polyps.[6] In contrast to AR, no evidence of T_H2-induced inflammation or epithelial barrier defects is found in patients with nonallergic, idiopathic rhinitis with nasal hyperreactivity,[66] indirectly suggesting that inflammation might be a crucial factor in induction of epithelial dysfunction.

Despite scientific breakthroughs in understanding the function of TJs, airway epithelial permeability in relation to pathology has

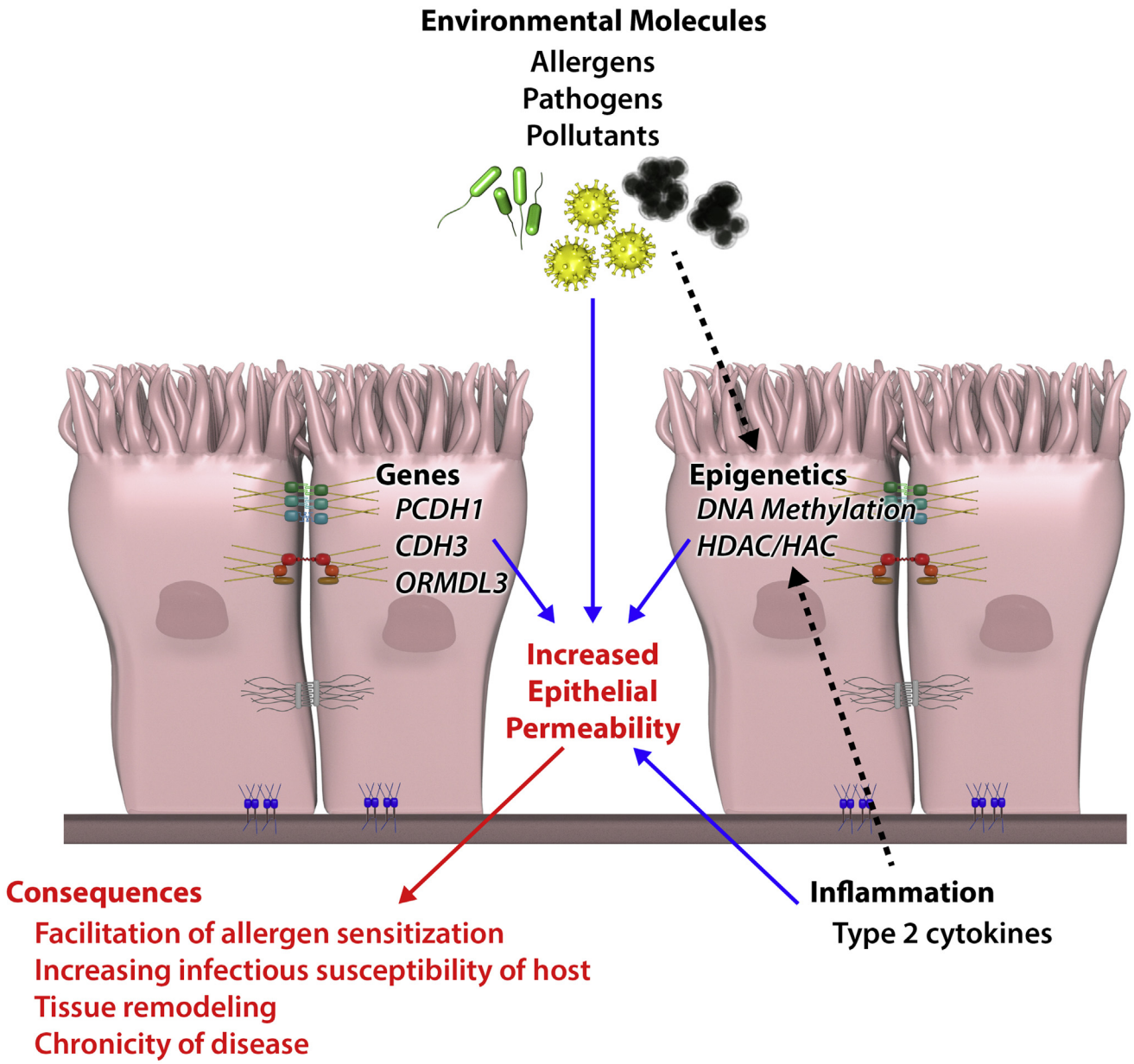

FIG 4. Overview of different mechanisms contributing to increased epithelial permeability in airway diseases. Environmental molecules and type 2 inflammation may damage epithelial integrity directly or via epigenetic changes induced in intercellular junction, resulting in increased permeability. Susceptibility genes, expressed in airway epithelial cells, may further enhance epithelial permeability. Consequently, allergen sensitization, tissue remodeling, and susceptibility of the host for pathogens is increased, resulting in chronicity of disease. *HAC*, Histone acetyltransferase.

not been studied extensively as an area of scientific interest for respiratory diseases. The heterogeneity of disease endotypes, the time-consuming and costly nature of primary epithelial cultures, and challenges in interpretation of *in vitro* study results[67] or translating murine findings to human diseases have all resulted in the lack of inclusion of nasal and bronchial epithelial barrier function in the guidelines for diagnostic work-out of patients affected by (allergic) rhinitis, CRS, and/or asthma.[68] The lack of commercially available tools to diagnose epithelial barrier dysfunction in the human airways, and the limited knowledge on the function of distinct epithelial cells, hampers the implementation of the airway epithelial barrier in clinical practice.

MECHANISMS CONTRIBUTING TO INCREASED EPITHELIAL PERMEABILITY

The mechanisms contributing to increased epithelial permeability are not fully clear. The dogma whether increased epithelial permeability is the result of environmental stress and/or inflammation or whether it is the result of genetic abnormalities is still standing. There is some evidence that epithelial stress and structural remodeling already starts at childhood and may contribute to the pathogenesis of asthma.[69-71] For now, we tend to accept that airway epithelial defects are caused by the combination of genetic traits and defects acquired by different internal and external factors[55,72] (Fig 4).

Effect of internal and external triggers on epithelial permeability

A wide variety of triggers are capable of disrupting the airway epithelial barrier function.[73,74] Allergens containing proteolytic activity, such as the major house-dust mite (HDM) allergen Der p1, can directly disrupt TJs and indirectly through the activation of protease-activated receptor-2.[75] Other environmental molecules such as viruses (rhinoviruses,[76] coronaviruses[77]) and pollutants (diesel exhaust,[78] cigarette smoke[79]) disrupt TJ and promote airway epithelium permeability, facilitating the induction of inflammatory processes. In turn, multiple cytokines are shown to interfere with TJs.[74] Our research group showed that type 2 cytokines IL-4 and IL-13 disrupt epithelial barrier integrity of nasal epithelial cells of patients with AR and healthy controls,[66] leading to a vicious cycle of increased epithelial permeability. Breaking this vicious cycle by limiting the exposure to environmental molecules, or by neutralizing cytokines, or restoring the increased epithelial permeability is a topic of great interest for airway diseases.

Genetic and epigenetic factors

Genome-wide association studies have identified several susceptibility genes associated with epithelial barrier function, differentiation, and homeostasis, including protocadherin-1 (PCDH1),[80] cadherin-related family member 3 (CDHR3), serine peptidase inhibitor, Kazal type 5 (SPINK5), and orosomucoid-like 3 (ORMDL3).[81] The exact contribution of these genes to epithelial barrier defects and airway inflammation is not completely understood. *PCDH1*, a susceptibility gene for bronchial hyperresponsiveness, belongs to the cadherin protein superfamily, and is a key component in preserving epithelial junctional structures.[82] siRNA-mediated knockdown of PCDH1 in 16HBE bronchial epithelial cells decreased TJ and adherence junction expression, leading to greater epithelial permeability,[83] both during establishment of barrier function and during epithelial repair after injury.[84] Lower *PCDH1* expression levels are found in inflammatory regions of nasal and bronchial epithelial cells of patients with CRS or asthma, respectively, compared with controls. Whether loss of PCDH1 contributes to epithelial barrier defects in patients with asthma carrying the *PCDH1* susceptibility gene, and whether there is an association with asthma severity, is not known. CDHR3 is identified as a susceptibility gene for recurrent severe exacerbations in patients with early childhood asthma. CDHR3 represents the docking site for rhinovirus C epithelial infection and replication.[85] The single nucleotide polymorphism rs6967330 in *CDHR3* is associated with higher protein expression, a 10-fold increase in binding and replication of human rhinovirus C, and a faster airway epithelial cell differentiation as indicated by increased ciliogenesis and more rapid development of functional cilia.[86] Recently, single-cell RNA sequencing on lung epithelial cells showed that CDHR3 was highly expressed in ciliated cells, but compared with mature cells, cadherin-related family member 3 expression was greater in immature, differentiation epithelial cells.[87,88] Using ***CRISPR-Cas9***, Everman et al[88] found that knockdown of cadherin-related family member 3 in primary epithelial cells did not affect the development or differentiation of ciliated cells but resulted in a decreased epithelial integrity. CDHR3 is a member of the cadherin family of transmembrane proteins and thus might influence vital processes, including barrier function. It is not clear, however, whether the reduced epithelial integrity caused by *CDHR3* knockdown is related to a reduced TJ function or to an effect on epithelial cells growth, leading to a reduced resistance. ORMDL3 is associated with early-onset asthma and fulfills a crucial role in sphingolipid homeostasis.[89] Several studies showed that ORMDL3 contributed to airway remodeling and inflammation.[90] Yang et al[91] showed that high levels of ORMDL3 attenuated claudin 17 and E-cadherin protein levels *in vitro* on primary bronchial and 16HBE bronchial epithelial cells, and in an asthma mouse model, whereas inhibition of ORMLD3 reconstituted epithelial barrier integrity.[91] This process was mediated via the activation of sphingosine kinase 1 and ERK, which are involved in TJ disruption.[92]

The genetic signature cannot only explain the increasing incidence and prevalence of airway diseases. The exposure to specific environmental or inflammatory triggers can play a key role in the induction or suppression of disease-related genes. Environmental and inflammatory triggers might affect gene expression through epigenetic mechanisms, thus priming immune and nonimmune cells with an allergic memory. ***Epigenetic regulation*** represents a secondary level of gene regulation that may result in tissue-specific stable changes in gene expression through 3 main mechanisms, including DNA modifications, histone modifications, and noncoding RNAs.[93] Changes in DNA methylation signatures are reported in epithelial cells of patients with asthma. Particularly, the basal cell marker, *KRT5*, is hypomethylated in patients with asthma.[94] Consequently, basal cell differentiation is dysregulated and results in epithelial barrier defects. HDM allergen challenge in mice resulted in aberrant methylation patterns of genes involved in the development of allergic asthma.[95] Recently, Forno et al[96] identified specific methylation profiles in epithelial cells that were associated with atopy and atopic asthma. Type 2 cytokines such as IL-13 are capable of altering DNA methylation patterns near asthma-associated genes and increase histone deacetylase activity in epithelial cells from patients with asthma and patients with AR.[97] The reversible acetylation or deacetylation of histone tails represents a secondary epigenetic regulatory mechanism. Acetylation of histone tails via histone acetyltransferases results in increased gene transcription, whereas removal of acetyl groups via histone deacetylases (HDACs) leads to gene suppression.[98] There are 18 HDAC isoforms, subclassified into 4 groups that vary in tissue distribution and subcellular localization.[99] We found increased endogenous HDAC activity in nasal epithelial cells of patients with AR, which was associated with epithelial integrity.[100] Expression of HDAC5 and HDAC11 mRNA and protein was enhanced in epithelial cells from patients, providing a potential explanation for increased HDAC activity. Interestingly, Wawrzyniak et al[101] reported increased expression of HDAC1 and HDAC9 in bronchial epithelial cells of patients with asthma, whereas Ito et al[102] reported decreased HDAC2 expression in epithelial cells from patients with COPD. The observation that multiple HDACs are changed in patients with asthma, AR, or COPD suggest a possible role for different HDACs in different disease phenotypes. IL-4 and IL-13 were shown to interfere with HDAC activity, showing that inflammation might induce long-lasting changes in epithelial cells.[101] Given that allergic memory, by means of chronic exposure to IL-4 and IL-13, imprinted in terminally differentiated epithelial and basal cells via epigenetic modifications,[28] we might speculate that airway epithelial cells are trapped in a diseased phenotype, contributing to disease chronicity. This theorem, however, is likely more complex than proposed here, and will need further confirmation.

TABLE I. Methods to evaluate airway permeability

Method	Human	Animal	Test molecule	Material needed	Advantages	Disadvantages
Histology						
	x	x	TJ expression, epithelial analysis	Biopsies, airway brushings	Disease-specific analysis	Invasive
Ex vivo						
Ussing chambers	x	x	FITC-dextran	Biopsies	Region-specific	Fresh material, invasive, labor-intensive
In vivo—permeability assays						
Albumin-^{125}I	x		Albumin-^{125}I	Serum	Sensitive, easy detection	Radioactive tracer
^{99m}Tc-DTPA	x		^{99m}Tc-DTPA	Serum	Sensitive, easy detection	Radioactive tracer
Mannitol	x		Mannitol	Serum	Marker for paracellular transport	Risk for bronchospasms
In vivo—biomarkers						
CC16	x		CC16	Serum	Easy detection	Indirect marker for epithelial damage
Zonulin	x		Zonulin	Sputum, nasal secretions	Information on epithelial permeability	ELISA sensitivity
In vitro						
Primary airway epithelial cells	x	x	FITC-dextran, etc	Biopsies, airway brushing	Patient and disease-specific	Cell culture variability, expensive, time-consuming

CC16, Clara cell secretory protein 16; *DTPA*, diethylenetriaminepentaacetic acid; *^{99m}Tc-DTPA*, diethylenetriamine-pentaacetate labeled with technetium 99; *FITC*, fluorescein-5-isothiocyanate.

CONSEQUENCES OF EPITHELIAL BARRIER DYSFUNCTION

There is increasing recognition that allergic airway diseases are not merely disorders of the immune system but rather a complex interplay between genes, environment, and lifestyle factors. Thus far, focusing on the immune component of allergic diseases did not result in therapies curing allergic diseases.[103] One attractive hypothesis relates to airway epithelial defects being the facilitator of allergic sensitization. Exposure to harmful stimuli, including internal, environmental, and/or allergenic components, may all hamper epithelial barrier function, leading to an increased access of harmful stimuli to the submucosal innate immune cells and blood vessels, ensuing sensitization and type 2–mediated inflammation (Fig 3).

Indeed, nasal epithelial barrier dysfunction has been shown to lead to increased access of particles to the submucosal compartment as well as in the systemic circulation.[55] Allergen inhalation has been reported to lead to mast cell degranulation only in those experimental settings with a deficient epithelial nasal epithelial barrier.[104] Interestingly, we observed that mast cell mediators rapidly contributed to increased epithelial permeability, which facilitated allergen penetration to the host.[66] In addition to mast cell mediators, also typical T_H2 cytokines IL-4 and IL-13 are released on allergen challenge of sensitized individuals and contribute to epithelial barrier dysfunction.[5,105] In contrast, strengthening the epithelial barrier reduced inflammation in different *in vivo* and *in vitro* models of T_H2-mediated respiratory inflammation.[106] We have shown that increased expression of TJs was associated with reduced mast cell degranulation and inflammation despite continuous exposure of the nasal mucosa to allergens.[104] In addition, in a mouse model of passive allergen sensitization, we observed increased mast cell activation when the epithelial barrier was compromised. It is therefore not hard to imagine that epithelial barrier dysfunction is a factor contributing to aggravation of mucosal inflammation. We may therefore speculate about nasal epithelial barrier dysfunction representing one of the crucial factors in the progression from upper to lower airways inflammation.[107] At present, rhinitis and CRS both represent risk factors for the development of asthma, with the hypothesis of barrier dysfunction being responsible in part for the chronicity of inflammation progress to lower airway inflammation. Systemic inflammation is part of the disease spectrum in AR, CRS, and asthma, and may represent a factor that contributes to the manifestation of inflammation in both upper and lower airways.

HOW TO MEASURE EPITHELIAL PERMEABILITY

Airway epithelial barrier defects are currently not routinely studied or quantified in clinical practice mainly due to the lack of a proper clinical test of "airway leakiness." Different techniques can be used to evaluate airway epithelial permeability (Table I). Mucosal biopsies or airway epithelial cells from upper and lower airways can be used for histological analysis of permeability and junctional proteins, though still requires an invasive intervention.[11] Mannitol, a small size molecule, has been used in dogs to measure changes in lung epithelial permeability.[108] Mannitol is well tolerated, migrates paracellularly across the epithelial barrier, and is not extensively metabolized. Recently, Georas et al[109] used inhaled mannitol for the evaluation of barrier function in asthma. Healthy controls and patients with mild asthma inhaled mannitol and serum levels of mannitol were determined afterwards. No difference in mannitol levels was observed between patients with asthma and controls, which might question the usefulness of inhaled mannitol as a marker for airway epithelial permeability. Alternative molecules that can be used for studying airway permeability are radioisotopes. Already in 1975, Buckle and Cohen[110] reported nasal mucosal hyperpermeability in atopic rhinitis and extrinsic asthma for nasally administered ^{125}I-Albumin compared with control subjects.[110] Furthermore, diethylenetriamine-pentaacetate labeled with technetium 99 has been used to assess lung permeability, though with discordant results. Some studies reported higher clearance of diethylenetriaminepentaacetic acid in lungs of patients with COPD and asthma,[111,112] whereas others reported similar clearance in patients compared with healthy subjects.[113] These discrepancies together with the radioactivity risks make these molecules less attractive, shifting the area toward the detection of biomarkers.

One potential biomarker of lung epithelial injury is the secretion of club cell secretory protein-16 (CC16) in sputum or bronchoalveolar lavage fluid.[114,115] CC16 has been studied in numerous airway diseases including asthma, COPD, idiopathic pulmonary fibrosis, and occupational- or environmental-induced lung injury.[115-117] The disadvantage of CC16, however; is that it is an indirect measure of epithelial damage, and not epithelial permeability. More recently, the serum protein zonulin has emerged as a popular biomarker to evaluate epithelial barrier function in chronic inflammatory diseases.[118] Zonulin was identified as a human endogenous modulator of epithelial TJs in the small intestine.[119,120] To our knowledge, there is only 1 report illustrating the involvement of zonulin in the regulation of lung permeability.[121] Whether zonulin can be used as a biomarker for increased airway epithelial permeability remains to be elucidated. No other biomarkers are currently proposed to measure increased epithelial permeability in upper and lower airway diseases. Large proteomic studies of nasal secretions, sputum, or bronchoalveolar lavage fluid are therefore warranted. Ideally, these biomarkers should be nondegradable, of suitable size to reflect epithelial permeability, and easily detectable.

Finally, there are promising results with novel imaging techniques, particularly ***confocal laser endomicroscopy***, to visualize cellular and interstitial structures in the airways.[122,123] With the development of novel contrast compounds and higher resolution optic fibers, molecular imaging might assist in the early diagnosis of airway diseases or might be used for the assessment of therapeutic outcome.

THERAPEUTIC CHALLENGES

Therapies targeting epithelial barrier defects might represent an attractive therapeutic strategy. A present, no Food and Drug Administration–approved therapies currently exist to restore or improve increased epithelial permeability in chronic airway diseases. However, several experimental approaches reconstituting epithelial barrier function *in vitro* and *in vivo* in mouse models have been reported.[11] These approaches focus on TJ regulation and have potential in preventing the onset of upper and lower airway pathology. We found that corticosteroids, a standard treatment option for asthma, AR, or CRS, improved TJ function and enhanced epithelial integrity *in vitro* and *in vivo* in an asthma mouse model.[11] The beneficial effect of corticosteroids on the epithelium is presumably induced via PCDH1.[83] PCDH1 expression is downregulated in nasal and bronchial samples of patients with CRS and patients with asthma.[124] In addition, corticosteroids decrease basal cell hyperplasia,[125] restoring the morphology of the airway epithelium.

Recently, therapies targeting epigenetic marks have been proposed as a novel strategy to repair epithelial barrier defects. Blocking HDAC activity enhances barrier function and junctional proteins in primary bronchial epithelial cells of patients with asthma[101] and in nasal epithelia cells of patients with AR.[100] In addition, Fukuda et al[126] showed that HDAC inhibition in cancer cells enhanced the expression of E-cadherin, which is important for epithelial-to-mesenchymal transition. Although the idea to interfere with epigenetics to promote epithelial barrier function seems attractive, the mechanisms are likely more complicated and potential side effects need to be investigated.[127]

Conclusions

Increased airway epithelial permeability is a key characteristic of chronic upper and lower airway inflammatory diseases. Whether barrier dysfunction precedes and predisposes to disease development is still not elucidated, but surely it maintains and contributes to the chronic nature of mucosal airway inflammation by facilitating paracellular transport of allergens, pathogens, and harmful stimuli. Developing functional and imaging techniques of epithelial barrier function will allow us to assess mucosal airway permeability *in vivo* and help identify patients who can benefit from barrier-restoring therapeutic plans. Preservation or reconstitution of airway barrier function is an intriguing novel domain of respiratory disease warranting extensive further studies.

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