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Airway epithelial barrier function regulates the pathogenesis of allergic asthma

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Abstract

The integrity of the airway epithelium in asthma patients is often disrupted, with loss of epithelial cell-cell contacts. Airway epithelial barrier dysfunction may have important

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implications for asthma, since structural epithelial barrier function is tightly interwoven with the ability of the epithelium to regulate the immune system. We propose that changes at the airway epithelial barrier play a central role in the sensitization to allergens and pathogenesis of allergic asthma. Many of the recently identified susceptibility genes for asthma are expressed in airway epithelium. However, the exact mechanisms by which the expression of epithelial susceptibility genes translate into a functionally altered response to aeroallergens in asthma is still unknown. In this review we will focus on the role of airway epithelial barrier function in the susceptibility to develop allergic asthma, and discuss therapeutic strategies aimed at the epithelial barrier.

Introduction

Asthma is a chronic inflammatory airway disease that affects up to 300 million people worldwide. Asthma patients suffer from symptoms of wheeze, sputum production, variable airflow limitation and airway hyperresponsiveness (AHR) to environmental bronchospasmogenic stimuli. Allergen-induced asthma is the most common type in the population and is characterized by elevated serum levels of immunoglobulin E (IgE), chronic eosinophilic airway inflammation, airway remodeling with increased smooth muscle mass, subepithelial fibrosis, epithelial desquamation and goblet cell hyperplasia. Type-2 T-helper (Th2) lymphocytes are key players in the airway inflammatory response of allergen-sensitized individuals, giving rise to the pathological changes and clinical symptoms of asthma. Susceptibility to allergic asthma has a strong genetic component, suggesting that the inception of the disease is the outcome of an interaction between genetic factors and environmental factors. Although allergic sensitization is known as the strongest identifiable predispositions for asthma, it cannot explain the prevalence of asthma alone. Of interest, a large number of newly identified asthma genes, including the

protocadherin family member *PCDH1* (1), are expressed in structural airway cells, particularly the epithelium, suggesting that processes at the mucosal surface of the airway are critical for the development of asthma (2).

The airway epithelium forms the first continuous line of defense against inhaled environmental insults, which include pathogens, pollutants and aeroallergens. Defective antimicrobial responses have been reported in bronchial epithelial cells from asthma patients (3). Furthermore, a number of observations suggest that the airway epithelium is disrupted in asthma. These include the detachment of columnar ciliated cells, presence of epithelial cell aggregates (creola bodies) in sputum, increased permeability to allergens and reduced expression of the epithelial cell-cell adhesion molecule E-cadherin. In addition, increased numbers of cells expressing the basal cell markers cytokeratin-5 and -14 and p63 (4), and increased expression of the repair markers TGF- β , EGFR and CD44 (5-7) have been demonstrated in the asthmatic airway epithelium. The defective epithelial barrier in the airways of asthmatic patients may have important consequences, leading to increased accessibility of allergens to immune and structural cells in the submucosa. Indeed, airway epithelial damage is known to correlate with more severe airway hyperresponsiveness (8). In addition, epithelial damage may result in increased epithelial pro-inflammatory activity and secretion of growth factors (2). Phenotypic changes and increased permeability in asthmatic airway epithelium have been related to enhanced NF- κ B activity and pro-inflammatory cytokine secretion upon exposure to environmental insults *in vitro* (4, 9). Biopsy studies in children suggest that structural changes in the airway epithelium may precede the initiation of airway inflammation, occurring early in asthma pathogenesis, even before definitive diagnosis (10). Moreover, a recent study suggests that structural epithelial alterations possibly caused by external insults rather than chronic inflammatory processes are crucial for the

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persistence of symptoms in certain individuals with severe asthma (11). Therefore, we propose that structural and functional abnormalities in the airway epithelium lead to aberrant signaling to the underlying immune and structural cells, propagating allergic airway remodeling, inflammation and hyperresponsiveness. However, the exact mechanisms by which the expression of epithelial susceptibility genes is translated into a functionally altered response to aeroallergens in asthma are still unknown. In this review we will focus on the role of airway epithelial barrier function in the susceptibility to develop asthma in response to aeroallergens. We propose that epithelial barrier function may be a novel therapeutic target, based on its central role in asthma pathogenesis.

Epithelial barrier function in asthma

Intercellular epithelial junctions form the structural adhesive forces of the airway mucosal barrier, separating the underlying tissue from the inhaled environment. These intercellular junctions are comprised of tight junctions (TJs), adherens junctions (AJs) and desmosomes. TJs are the main regulators of paracellular permeability and movement of ions and solutes between cells. Transmembrane proteins of TJ include junction-adhesion-molecules (JAM)s, occludin, and claudins that anchor to the cytoskeleton via zona occludens-(ZO)-1, -2, -3 and cingulin. At the basolateral site of TJs, AJs mechanically connect adjacent cells and initiate proliferation and differentiation through homotypic transmembrane E-cadherin adhesions, which are anchored to the actin cytoskeleton and microtubule network by p120 catenin, β -catenin, and α -catenin. Desmosomes consist of non-classical cadherins that form adhesive bonds between the filamentous cytoskeleton of epithelial cells and the lamina propria (2). Specifically E-cadherin plays a crucial role in AJs as well as TJs, providing the architectural support required to form

these junctional complexes, and delocalization of TJ proteins ZO-1, occludin and claudins occurs following distortion of AJ architecture (12, 13).

Studies on airway epithelium junctional proteins in asthmatic patients have reproducibly shown that expression of ZO-1, E-cadherin and occludin are downregulated in the epithelium of asthmatics *in vivo* as well as *in vitro*, suggesting a broad defect in adhesion mechanisms (4, 14-16). This phenomenon is also present in the upper airways, as disrupted desmosome formation has been observed in nasal polyps from asthmatic children (17). Epithelial barrier damage may be due to direct cleavage of epithelial junctions via allergen proteolytic activity, downregulation of junctional protein expression through inflammatory mediator release, or dysregulation of E-cadherin membrane trafficking by downregulation of caveolin-1 (18-21). Caveolin-1, a scaffolding molecule present in specific membrane microdomains (caveolae), regulates epithelial barrier function by stabilization of E-cadherin-mediated cell-cell contacts, and displays reduced expression in asthma epithelium (21, 22). This may render airway epithelial cells from asthma patients more susceptible to allergens. Although the mechanisms underlying the loss of E-cadherin-caveolin-1 membrane complexes are currently unknown, genetic variation may increase epithelial vulnerability to environmental insults. Many asthma candidate genes identified by genome-wide association studies are expressed within the respiratory epithelium, as outlined below. Recent findings indicate that asthma epithelium is more prone to cigarette smoke (16) as well as allergen-induced disruption of cell-cell contacts, the latter of which may be mediated by aberrant Ca^{2+} signaling (23). Bronchial epithelial cells from asthma patients were more susceptible to HDM-induced Ca^{2+} influx and subsequent barrier dysfunction (23).

Alternatively, loss of airway epithelial integrity in asthma may be due to inefficient repair and regeneration into fully differentiated and functionally intact epithelial layer upon exposure to

environmental insults, including allergens and viral infection. This is supported by increased expression of repair markers (TGF- β , EGFR) (5, 6) and a more basal epithelial phenotype with increased expression of CK-5 and p63 of asthma epithelium *in vivo*, and decreased differentiation with lower E-cadherin expression upon air-liquid culture *in vitro* (4, 16).

Asthma risk factors: Environmental exposures acting on airway epithelium

Birth cohort studies indicate that both early-life sensitization to aeroallergens and lower respiratory viral infections are important risk factors for asthma in children, and the highest risk for progression to persistent asthma occurs when these two exposures coincide in early life (7). Remarkably, aeroallergen sensitization was shown to lead to more severe virus-induced lower respiratory illness (24). Two highly frequent respiratory viruses, rhinovirus (RV) and respiratory syncytial virus (RSV), are thought to play a major role in asthma pathogenesis upon binding to specific receptors on the surface of the airway epithelium. RV for instance binds to ICAM-1 (major group) or low density lipoproteins (LDLs, minor group) on the surface of the airway epithelium (25). This causes viral endocytosis, and once inside host epithelial cells, viruses uncoat and initiate the viral replication process. The interaction between virus and epithelial cells leads to the stimulation of immune receptors and the production of type-I interferons (IFNs) to kill invading pathogens and stimulate inflammation and adaptive immunity by the release of pro-inflammatory cytokines that drive DC activation (26, 27). Subsequent humoral and DC-induced cell-mediated immunity results in the generation of B cells, CD4⁺ T helper and CD8⁺ cytotoxic T cells that further contain the infections. However, the presence of underlying chronic inflammatory conditions, such as asthma, alters immunity to pathogens that may undermine the protective effects of the airway epithelium. In asthma, high levels of IL-13 are present, which

suppresses epithelial pathogen clearance (28, 29). Furthermore, impaired epithelial barrier function in asthma is accompanied by reduced IFN responses, impairing the innate immune response to infection (3, 30). These deleterious responses may be reinforced by aberrant responses to infection that increase inflammatory and type-2 responses (31, 32). Exposure of airway epithelial cells to double-stranded RNA or infection with RV or RSV *in vitro* drives a marked upregulation of TSLP expression. These findings may help explain why respiratory viruses exacerbate allergic inflammation despite inducing type-1 immune responses that could theoretically counterbalance type-2 mediated inflammation.

RV-associated wheezing illness during infancy is strongly related to asthma at school age (10). Data from the COAST study show that allergic sensitization precedes RV-induced wheezing, while the reverse was not observed (24), indicating that RV-induced disease follows aeroallergen sensitization. A recent combined study from the COAST and COPSAC studies demonstrates that association of known asthma genes at the 17q21 locus with the disease was restricted to children with RV-associated wheezing illness during infancy (33). This indicates that aeroallergen sensitization alters the response to lower respiratory infection and onset of the disease process in children. Furthermore, RSV bronchiolitis during infancy is strongly related with subsequent wheeze (34), and also associated to asthma, albeit to a lesser extent than RV. Monoclonal antibodies to RSV prevent recurrent wheeze in the first year of life in susceptible late preterm born children, strongly suggestive of a causal role of RSV infection in this phenotype (35) that is associated with increased risk of asthma.

Of note, exposure to both allergens and virus is known to reduce epithelial barrier function. Exposure of airway epithelial cells *in vitro* to proteolytically active allergens such as Der p1 derived from house dust mite (HDM) (18, 36), ragweed, white birch, grass and pollen

(37), can lead to the proteolytic degradation of airway epithelial intercellular adhesions by cleavage of the junctional proteins. HDM, cockroach, fungi and mold extracts are also thought to indirectly degrade AJs via activation of PAR-2 (38, 39). Exposure of human airway epithelial cells to HDM induces rapid, transient reduction in epithelial resistance, concomitant with relocalization of E-cadherin and ZO-1. However, recent data indicate that HDM-induced bronchial epithelial barrier dysfunction is mediated independently of serine and cysteine proteases (40). Instead, this may involve Ca^{2+} /calpain-dependent disruption of epithelial junctions (23). Similar to allergens, RV and RSV affect epithelial integrity and reduce expression of epithelial junction proteins (2). Rhinovirus (RV) reduces transepithelial resistance via loss of ZO-1 from TJs, and increases airway epithelial permeability (41). *In vivo* sensitization and challenge with ovalbumin in mice causes disruption of TJs as well as other gap junction proteins such as connexin 37 in lung epithelial cells (42).

Expression of asthma susceptibility genes in the airway epithelium

As outlined above, asthma is likely the outcome of an interaction between genetic factors and environmental exposures, leading to the disruption of epithelial barrier function. The heritability of the susceptibility to the disease is about 50-60% (43). Genetic susceptibility to asthma is dependent on a large number of genes that have been identified over the years by different approaches (44). In addition to polymorphisms, epigenetic mechanisms contribute to heritable changes in gene expression that contribute to asthma susceptibility (45). Unbiased genetic screens such as positional cloning and genome-wide association (GWA) analyses, making no prior assumptions regarding the relevant mechanisms in disease pathogenesis or asthma gene

identity, can be expected to reveal those genes that most faithfully reflect the mechanisms critical to the disease (46). Of note, a large number of asthma genes identified by unbiased approaches are expressed in the airway epithelium, including PCDH1 (1), CDHR3 (47), ORMDL3, SMAD3, HLA-DR, IL1RL1 and IL-33 (48). Recent studies have clearly demonstrated that epithelial derived cytokines, including IL-33, IL-25 and TSLP are upstream of classical type-2 cytokines such as IL-4, IL-5 and IL-13 that are involved in allergic inflammation (49-54). The ontology of these asthma genes seems to indicate the existence of two central themes conferring asthma susceptibility, (i) activation of an innate immune response leading to type-2 immunity and (ii) integrity and repair of the airway epithelium. Regarding the activation of an innate immune response, *IL1RL1* is the most replicated asthma gene, harboring a number of discrete genetic signals that independently contribute to several asthma phenotypes in multiple populations, underscoring its relevance to the pathogenesis of the disease (45). *IL1RL1* encodes the receptor for IL-33, and is expressed on the airway epithelium as well as on a wide range of cells of the innate and adaptive immune system, including type-2 innate lymphoid cells (ILC2s). IL-33 is expressed in the airway epithelium and released upon damage of the cells, thereby inducing an innate immune response. Interestingly, the *RORA* gene, encoding a transcription factor critical for ILC2 differentiation, was identified in the GABRIEL consortium GWA analysis with near-genome wide significance (48). Since *RORA* expression is induced by IL-33 upon IL1RL1 binding, this seems to identify activation of the type-2-biased innate immune response upon epithelial damage as a central pathway in asthma pathogenesis (45). Additionally, there is considerable evidence implicating polymorphisms in the TSLP gene in the pathogenesis of inflammatory airway diseases. Substantially elevated levels of TSLP are found in airway biopsies of asthmatics (55) and lung-specific transgenic expression of TSLP is sufficient to

induce features of asthma in a murine model including airway inflammation, infiltration by eosinophils, goblet cell hyperplasia and AHR (56, 57).

Most of the asthma susceptibility genes expressed in the airway epithelium likely contribute to epithelial integrity or repair thereof upon environmental challenges. Several of these encode adhesion molecules and are thought to directly regulate airway epithelial integrity, including PCDH1 (58) and CDHR3 (47). ORMDL3 regulates cytosolic Ca^{2+} entry by the sarco-endoplasmic reticulum (ER) Ca^{2+} ATPase (SERCA) pump, which was recently shown to be involved in HDM-induced epithelial barrier dysfunction (23). SMAD3 is a signal transduction molecule for the pro-fibrotic cytokine and repair marker TGF- β . The TGF- β /Smad3 pathway has been found to contribute to the airway epithelial response to both aeroallergen exposure (59, 60) and respiratory viral infection (61-63), increasing replication of both RSV (61, 62) and RV (63) in primary bronchial epithelial cells. Bronchial epithelial cells from asthma patients were found to produce higher levels of TGF- β , contributing to the enhanced RV replication (63). Furthermore, TGF- β /Smad3 signaling plays a role in epithelial plasticity during repair responses, as further outlined below. Thus, alterations in these epithelium-expressed susceptibility genes may contribute to an altered response to environmental insults, with loss of epithelial integrity, propagating the disease process.

Consequences of loss of epithelial cell-cell contacts and barrier function

We propose that the compromised epithelial barrier function in asthma is not secondary but causal to the disease pathogenesis, having important implications for the development of allergic asthma. The first direct link between compromised epithelial barrier function and allergy has been provided by findings in atopic dermatitis. Here, mutations in the epidermal barrier protein filaggrin (*FLG*) gene, leading to a defect in epithelial barrier function, have been associated with

increased epithelial permeability to environmental insults and enhanced allergic sensitization in the form of asthma and atopic dermatitis (64, 65). Similar processes may be involved in sensitization of the airways to aeroallergens. Mutations in E-cadherin and PCDH1 genes are associated with airway hyperresponsiveness (ARH), suggesting that defects in barrier function may drive the type of response to antigen exposure (1, 66).

As described above, loss of epithelial barrier function has been associated with inflammatory responses, resulting in enhanced NF- κ B activity and increased pro-inflammatory cytokine production (4, 9). Of interest, the induction of E-cadherin expression is known to suppress the activity of NF- κ B in mesenchymal cells (67), while EGFR-dependent NF- κ B activation is critically involved in HDM-induced production of the Th2 cell-attracting chemokine CCL17 by airway epithelial cells (68). Following epithelial injury, exposure to inflammatory cytokines (IL-1 β , TNF, IL-4 and IL-13) enhances the secretion of TSLP from airway epithelial cells (69-72). In line with a role for epithelial cell-cell contacts in the regulation of pro-allergic immune responses, siRNA downregulation of E-cadherin resulted in EGFR and EGFR-dependent expression of Th2 cell chemo-attractant CCL17 and Th2-driving factor TSLP in airway epithelium (73). Recently, TSLP expression was also linked to alterations in the expression of E-cadherin membrane stabilizing protein caveolin-1 (21). Expression of caveolin-1 was significantly lower in airway epithelium from asthma patients than non-asthmatics, a finding that persisted both in long term submerged and air-liquid interface culture systems. Reduced caveolin-1 expression was accompanied by a reduction in E-cadherin expression, disrupted barrier function and increased levels of TSLP. Studies in air-liquid interface (ALI)-differentiated human bronchial epithelial cells from asthma and control subjects have shown that reduced expression of E-cadherin is accompanied by increased levels of TSLP at baseline (21)

and a stronger induction of pro-inflammatory cytokines IL-6, IL-8 and GM-CSF upon exposure to virus, particulate matter or mechanical wound (4). The role of E-cadherin-mediated cell-cell contacts in the regulation of allergic airway inflammation is further supported by findings in a mouse model of asthma (40). Here, the ability of HDM extract to disrupt airway epithelial expression of E-cadherin was related to allergic sensitization and the development of inflammatory inflammation, with increased CCL17 and Th2-type cytokine production in response to the extract. In addition to homotypic cell-cell contacts, E-cadherin is a ligand for the cognate receptor CD103 ($\alpha_E\beta_7$ integrin) and killer cell lectin-like receptor G1 (KLRG1) on innate and adaptive immune cells. CD103 is expressed on CD8⁺ T cells, a significant fraction of effector CD4⁺ T cells and regulatory CD4⁺CD25⁺Foxp3⁺ T cells (Tregs) (74). CD103 also identifies a novel subset of dendritic cells (DCs) that also express E-cadherin, TJ proteins and langerin (75), are involved in tolerance induction of following inhaled allergen (76), and are critical for the clearance of several respiratory viral infections (75-78). KLRG1 is an inhibitory receptor expressed on a subset of activated natural killer (NK) cells, effector/memory T cells and Foxp3⁺ Tregs. Engagement of KLRG1 inhibits secretion of inflammatory cytokines by DCs, thereby exerting immunosuppressive effects (79). Thus, the expression of adhesion proteins such as E-cadherin on intact epithelium may also play a significant role in regulating inflammation through the inhibition of DC and T cell activation.

In addition to the regulation of inflammatory responses, loss of epithelial barrier function may act to promote tissue remodeling. Especially when damaged, the epithelial layer communicates to the underlying mesenchyme by production of growth factors and matrix metalloproteinases (MMPs), acting on mesenchymal cells and extracellular matrix (ECM) to regulate tissue repair and remodeling processes (2). Furthermore, loss of E-cadherin-mediated

cell-cell contacts and subsequent liberation of β -catenin from cell-cell contacts is an important aspect of epithelial plasticity or epithelial-to-mesenchymal transition (EMT), a process involved in cell migration, repair and tissue remodeling (80). β -catenin and the transcriptional repressors of E-cadherin, Snail family members Sna-1 and Sna-2/Slug, Twist and ZEB1 are key regulators of the EMT program (2, 81, 82). During EMT, cells lose their epithelial characteristics and gain expression of mesenchymal markers, including vimentin and fibronectin, myofibroblast marker smooth muscle actin (α -SMA), ECM proteins, e.g. periostin, MMP-2, -7 and -9, growth factors e.g. VEGF, and EGFR (2, 83). Multiple studies suggest a role for EMT in the cellular pathology of asthma (83-86); in addition to E-cadherin and cytokeratin loss, increased expression of several mesenchymal markers, including fibronectin, α -SMA and periostin has been observed during re-epithelialization after tissue damage and/or during chronic inflammatory conditions, e.g. asthma (2, 22). Periostin is of specific interest, as this EMT marker has recently emerged as biomarker of airway eosinophilia in asthma (87). In addition to binding $\alpha_M\beta_2$ integrin on eosinophils to promote their migration, periostin can promote epithelial expression of MMP-2, MMP-9 and collagen in a TGF- β -dependent manner (88, 89). TGF- β , which plays a well-established role in airway remodeling in asthma, induces the expression of EMT markers to a broader extent in bronchial epithelial cultures from asthma patients than healthy controls (85). HDM has been shown to promote TGF- β -induced EMT in bronchial epithelial cells *in vitro* and to induce characteristics of EMT in a mouse model of asthma. Here, the contribution of EMT to airway remodeling was established by cell-fate mapping of airway epithelial cells, which were found to migrate to subepithelial layers of the airway wall. Together, epithelial damage with loss of E-cadherin-mediated cell-cell contacts may induce an epithelial phenotype that promotes both inflammatory responses and abnormal repair and remodeling responses in the airways.

Therapeutic strategies to improve barrier function

Targeting the epithelial barrier may constitute a novel therapeutic strategy to ameliorate disease severity in asthma. Regarding the damaging effects of allergens on the epithelial barrier, recent findings suggest indicate that targeting Ca^{2+} signaling, for instance by the pharmacological calcium antagonists, may be beneficial. As mentioned above, Ca^{2+} signaling was shown to be involved in HDM-induced disruption of bronchial epithelial barrier function (23). Many studies have previously shown bronchodilator effects of calcium channel blocker/antagonist nifedipine, attenuating airway responsiveness especially in exercise-induced asthma, although it nifedipine failed to reduce signs and symptoms in persistent asthma (90). Thus, different strategies may be required to reverse the chronic structural changes in the airway epithelium and subepithelial layers in asthma. As outlined above, caveolin-1 may be a promising molecular target in the improvement of epithelial integrity (21). A novel drug based on the caveolin-1 scaffolding domain peptide can restore caveolin-1 function to cells deficient in caveolin-1, which has proven to be effective *in vitro* (reversing fibroblast collagen expression) and *in vivo* (reversing inflammation and fibrosis in bleomycin-treated mice) (22). However, to our knowledge no effects on epithelial barrier function have been described and the peptide has not yet been used in clinical trials. Additionally, corticosteroids can induce the expression of caveolin-1 in lung epithelial cells (91). Although corticosteroids failed to prevent the TGF- β -induced downregulation of E-cadherin in a bronchial epithelial cell line (92), recent findings in primary bronchial epithelial cells have shown that inhaled corticosteroids (ICS) protect against cigarette smoke-induced barrier dysfunction (93). However, asthma epithelium was found less responsive to the protective effects of ICS (93). Therefore, strategies to restore ICS sensitivity could be beneficial in improving epithelial barrier function in asthma combination with ICS, including the

use of antioxidants, pharmacological inhibition of PI3K or α -IL-17 antibodies or (94). Oxidative stress has been implicated in corticosteroid unresponsiveness (95, 96), leading to PI3K-dependent post-translational histone deacetylase 2 (HDAC2) modifications and proteasomal HDAC2 degradation (97, 98). HDAC2 deacetylates the glucocorticoid receptor as well as histones at NF- κ B response elements within promoter regions of inflammatory genes (99, 100). Additionally, IL-17 was shown to reduce steroid responsiveness in human bronchial epithelial cells in a PI3K-dependent manner (101). Thus, preventing oxidative stress-mediated, PI3K-dependent degradation of HDAC2 may restore epithelial responsiveness to corticosteroids and their protective effect on epithelial barrier function (93). In addition, regarding TGF- β -induced changes in epithelial phenotype, Smad3 may be a target of interest to improve epithelial barrier function in asthma, especially since this is also an asthma susceptibility gene. Smad3 has been implicated in EMT, and siRNA down-regulation of Smad3 was shown to attenuate the TGF- β -induced down-regulation of E-cadherin in ALI-cultured primary bronchial epithelial cells (85). Accordingly, TGF- β can upregulate the expression of Smad3-dependent E-cadherin repressors Snai-1 and -2 (85). Activation of ERK can induce Smad3 promoter activity in epithelial and smooth muscle cells, and ERK and p38 MAPK have been shown to induce E-cadherin down-regulation (102-104). However, specific p38 and ERK-1/2 inhibitors failed to prevent TGF- β -induced E-cadherin expression in primary bronchial epithelium (85). *In vivo*, SD-208, inhibitor of TGF- β receptor I (TGF- β RI) kinase reduced Smad-2/3 phosphorylation, epithelial proliferation, goblet cell hyperplasia, recruitment of eosinophils and T cells and OVA-specific IgE in an OVA rat model, although it did not significantly decrease smooth muscle thickness (105). Nevertheless, Smad3 siRNA down-regulation not only reversed the TGF- β -induced down-

regulation of E-cadherin, but also the expression of mesenchymal markers, including fibronectin, in human bronchial epithelium *in vitro* (85).

Concluding remarks

The expression of many asthma susceptibility genes within the airway epithelium indicates that it plays a central role in the pathogenesis of asthma. The airway epithelial barrier is often disrupted in asthma, and specifically polymorphisms in *PCDH1* and *SMAD3* genes may affect epithelial barrier function, increasing epithelial vulnerability to environmental insults, including allergens and viruses. This may result in aberrant signaling between epithelial and immune cells as well as mesenchymal cells, promoting especially Th2-driven airway inflammation, aberrant repair, and EMT/remodeling responses. Future studies in conditional E-cadherin knock-out mice will provide further insight into the role of epithelial cell-cell contacts in allergic airway inflammation. We propose that epithelial cell-cell contacts may constitute a novel target for the treatment of asthma, based on its various roles in mucosal permeability to allergens, inflammatory responses and airway remodeling. Drugs to restore caveolin-1 function, improve steroid responsiveness or inhibit of pathways involved in EMT, including TGF- β /Smad3 signaling, may be promising.

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Figure 1. Overview of the role of epithelial barrier function and immunological function in the pathogenesis of asthma. A number of environmental exposures, including house dust mite (HDM) and viruses can induce disruption of the epithelial barrier. Susceptibility genes expressed in the airway epithelial may enhance the vulnerability to environmental insults and/or disturb epithelial repair and re-differentiation into a functionally intact layer with tight cell-cell contacts. Reduced expression of specifically E-cadherin may result in Epidermal Growth Factor Receptor (EGFR)-dependent signaling and increased production of pro-inflammatory cytokines, including TSLP, CCL17 and GM-CSF, promoting type-2 T-helper (Th2)-driven responses and activation/attraction of granulocytes. In addition, loss of E-cadherin may result in altered epithelial repair and plasticity in a process referred to as epithelial-to-mesenchymal transition (EMT), resulting in increased expression of mesenchymal markers and matrix metalloproteases (MMPs). Furthermore, epithelial damage may lead to release of growth factors, e.g. TGF- β , promoting airway remodeling. Inhaled corticosteroids (ICS) suppress epithelial pro-inflammatory activity and may also act to improve epithelial barrier function.

