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Clinical Immunology

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REVIEW

Airway epithelial regulation of pulmonary immune homeostasis and inflammation



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Received 19 October 2013; accepted with revision 4 December 2013

Available online 14 December 2013

KEYWORDS

Asthma;
Epithelium;
Chemokine;
Chronic-obstructive
pulmonary disease;
Host defense;
Tight junctions

Abstract Recent genetic, structural and functional studies have identified the airway and lung epithelium as a key orchestrator of the immune response. Further, there is now strong evidence that epithelium dysfunction is involved in the development of inflammatory disorders of the lung. Here we review the characteristic immune responses that are orchestrated by the epithelium in response to diverse triggers such as pollutants, cigarette smoke, bacterial peptides, and viruses. We focus in part on the role of epithelium-derived interleukin (IL)-25, IL-33 and thymic stromal lymphopoietin (TSLP), as well as CC family chemokines as critical regulators of the immune response. We cite examples of the function of the epithelium in host defense and the role of epithelium dysfunction in the development of inflammatory diseases. © 2014 Elsevier Inc. All rights reserved.

Abbreviations: AAM, alternatively activated macrophage; AEC, airway epithelial cell; AHR, airway hyperresponsiveness; AJ, adherens junction; CARD, caspase recruitment and activation domain; CPA3, carboxypeptidase A3; DAMP, damage associated molecular pattern(s); DC, dendritic cell; EMCV, encephalomyocarditis virus; HNP, human neutrophil defensin; FLG, filaggrin; HDM, house dust mite; ICAM-1, intercellular adhesion molecule 1; IFN, interferon; IL, interleukin; JAM, junction adhesion molecule; KLRG1, killer cell lectin-like receptor G1; MAPK, mitogen activated protein kinase; LDL, low density lipoprotein(s); MC, mast cell; MDA5, melanoma differentiation-associated protein 5; MID1, midline 1; NK, natural killer; NLR, NOD-like receptor; PAMP, pathogen-associated molecular pattern(s); PAR, protease-activated receptors; PRR, pattern-recognition receptor(s); RIG-I, retinoic acid-inducible gene 1; RLR, RIG-I-like receptor; RORA, retinoic acid-related orphan receptor α ; RSV, respiratory syncytial virus; RV, rhinovirus; SP-C, surfactant protein C; STAT, signal transducer and activator of transcription; TJ, tight junction; TLR, Toll-like receptor; Treg, regulatory T cell; TSLP, thymic stromal lymphopoietin; TSLPR, TSLP receptor; ZO, zonular occluden.

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1. Introduction

The regulation of immune homeostasis in the lung has long focused on leukocytes as the key orchestrators of the immune system; however, genetic, structural, and functional studies indicate that the epithelium is required for normal immune homeostasis and that epithelial dysfunction is involved in the development of inflammatory disorders of the airways and lung. The airway epithelium functions an essential barrier that responds to environmental stimuli as a critical immune regulator through the secretion of cytokines, chemokines, growth factors, antimicrobial peptides, and the recruitment of leukocytes. This epithelial centered view of disease pathogenesis provides a better understanding of the reasons why diverse stimuli such as inhaled allergens and non-allergic triggers such as pollutants, cigarette smoke, bacterial peptides, and viruses can all drive characteristic immune responses through their interactions with the epithelium. The epithelium is ideally situated to orchestrate and influence the adaptive immune response, and in this capacity acts as an interface between innate and adaptive immune regulation. In this regard, epithelial production of interleukin (IL)-25, IL-33 and thymic stromal lymphopoietin (TSLP), as well as CC family chemokines have emerged as critical epithelial factors that can initiate and amplify airway inflammation. These newly identified epithelial-specific cytokines are produced prior to the release of more commonly recognized Th2 type mediators of allergic inflammation including IL-4, IL-5 and IL-13. The objective of this article is to review the recent findings about the molecular and cellular mechanisms by which the epithelium regulates airway inflammation, host defense and immunity. We focus on the impact of inhaled environmental stimuli and pathogens on epithelial cell function and the role of these cells in immune regulation.

1.1. The epithelial barrier

The airway epithelium forms a continuous, highly regulated physical barrier that lines the airway lumen, separating the underlying tissue from inhaled environmental antigens. Intercellular epithelial junctions form the structural adhe-

sive forces that maintain the airway epithelial barrier, and are comprised of tight junctions (TJs), adherens junctions (AJs) and desmosomes (Fig. 1). Tight junctions are the main regulators of paracellular permeability and movement of ions and solutes between cells. Transmembrane proteins such as junction-adhesion-molecule (JAMs), occludin, and claudins that anchor to the cytoskeleton to zonular occluden (ZO)-1, -2, and -3 and cingulin form these tight junctions. Adherens junctions mechanically connect the adjacent cells and initiate proliferation and differentiation through homotypic transmembrane E-cadherin adhesions that are anchored to the actin cytoskeleton and microtubule network by p120 catenin, β -catenin, and α -catenin. E-cadherin provides the architectural support required to form other junctional complexes, as delocalization of the TJ proteins ZO-1, occludin and claudins occurs following distorted AJ architecture [1,2]. Desmosomes consist of non-classical cadherins that form adhesive bonds between the filamentous cytoskeleton of epithelial cells and the lamina propria. In addition to its role in AJs, E-cadherin is also a ligand for the cognate receptor CD103 ($\alpha_E\beta_7$ integrin) expressed on innate and adaptive immune cells, including CD8⁺ T cells, and a significant fraction of effector CD4⁺ T cells and regulatory CD4⁺CD25⁺Foxp3⁺ T cells (Tregs) [3]. CD103 also identifies a novel subset of dendritic cells (DCs) that also express E-cadherin, TJ proteins and langerin [4] that are involved in the induction of tolerance following inhaled allergen [5], and are critical for the clearance of several respiratory viral infections [4–7]. E-cadherin also binds to killer cell lectin-like receptor G1 (KLRG1), 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 [8]. Thus, it is tempting to speculate that the interaction of innate and adaptive immune cells with E-cadherin on intact epithelium may play a role in the inhibition of DC and T cell activation.

Exposure of airway epithelial cells (AEC) *in vitro* to proteolytically active allergens such as Der p1 derived from house dust mite (HDM) [9,10], or Ragweed, White Birch, Kentucky Blue Grass, and Easter Lily pollen [11] can lead to the proteolytic degradation of airway epithelial intercellular

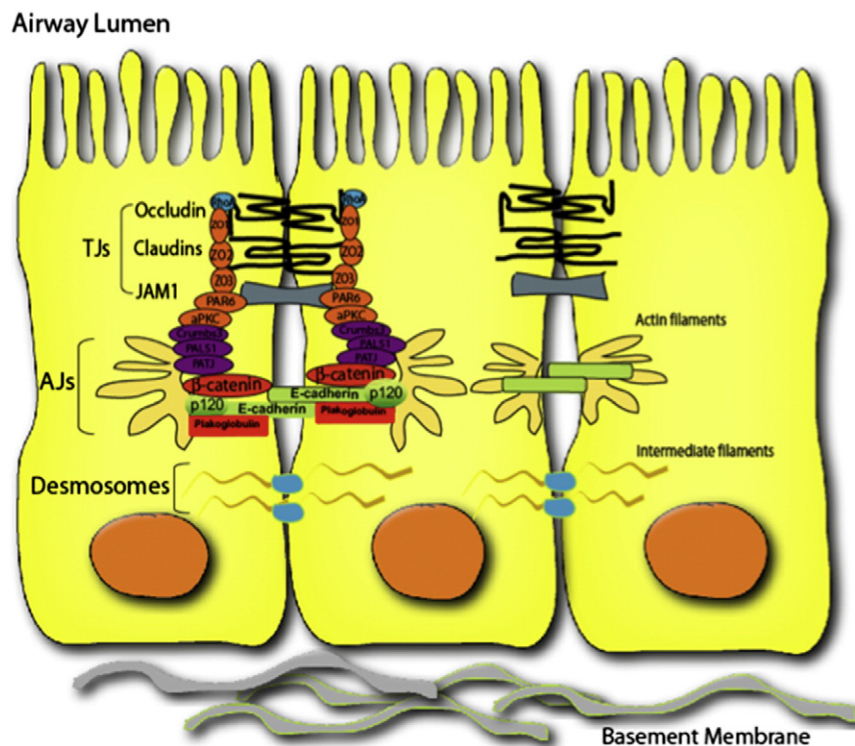


Figure 1 Cell to cell adhesion junctions in the airway epithelium. Tight junctions (TJs) are composed of the interacting transmembrane proteins junction-adhesion-molecule (JAMs), occludins, and claudins which are anchored to the cytoskeleton by zonular occluden (ZO)-1, -2, and -3 and cingulin. JAMs bind the cell polarity proteins Par-3 and -6, while occludin and claudins are anchored to the cytoskeleton by ZO-1, -2, and -3 and cingulin. Claudins function to regulate the permeability of the plasma membranes between adjacent cells, whereas occludin is involved in the regulation of *de novo* assembly of TJs. Adherens junctions (AJs) mechanically connect adjacent cells and initiate the formation and maturation of cell–cell contacts through homotypic, calcium-dependent adhesions of E-cadherin, a type I cadherin transmembrane glycoprotein, which is stabilized in the membrane by binding to anchor proteins p120 catenin, β -catenin, and α -catenin, which form an interface with the cell's microtubule network and actin cytoskeleton. Desmosomes consist of non-classical cadherins and form adhesive bonds with the filament cytoskeleton of the cells, such as the armadillo proteins plakoglobin and plakophilin, and desmoplakin interacts with intermediate filaments such as cytokeratin-5.

adhesions. HDM, cockroach, fungi and mold extracts are also thought to indirectly degrade AJs *via* activation of PAR-2 [12,13]. Exposure of human AECs to HDM induces rapid, transient reduction in epithelial resistance, concomitant with relocalization of E-cadherin and ZO-1, the latter through proteolytic degradation. Rhinovirus (RV) also reduces trans-epithelial resistance *via* loss of ZO-1 from TJs, and increases AEC permeability [14]. *In vivo* sensitization and challenge with ovalbumin in mice causes a disruption of TJs as well as other gap junction proteins such as connexin 37 in lung epithelial cells [15]. The transepithelial migration of neutrophils is highly dependent on the cleavage of the intracellular domains of E-cadherin and occludin by the endogenous protease calpain [16]. Further, granulocytes also cause a disruption of cell–cell contacts in nasal epithelium *in vivo*, with eosinophils inducing the most pronounced effects [17].

The few studies on AEC junction proteins in asthmatic patients have reproducibly shown that expressions of ZO-1, E-cadherin and occludin are downregulated in the epithelium of asthmatics, suggesting a broad defect in adhesion mechanisms [18–21]. This phenomenon is also present in the upper airway as disrupted desmosome formation is found in nasal polyps from asthmatic children [22]. The mechanism of epithelial barrier

damage may be due in part to the downregulation of junction proteins through inflammatory mediator release, directly *via* allergen proteolytic activity, or through dysregulation of membrane trafficking by downregulation of caveolin-1 [9,23–25]. Genetic variation may also play a role as many novel asthma genes identified by genome-wide association studies are expressed within the respiratory epithelium. Mutations in E-cadherin and protocadherin 1 genes are also associated with airway hyperresponsiveness (ARH), suggesting that defects in barrier function may drive the type of response to antigen exposure [26,27]. For example, genetic variants of the epithelial protein filaggrin (FLG) lead to defects in epithelial barrier function as well as enhanced allergen sensitization in the form of asthma and atopic dermatitis [28,29].

2. The epithelium as a sensor of environmental danger signals

With each breath, inhaled antigens are deposited on the tracheal and bronchial epithelium, and are normally cleared by mucociliary transport or removed by phagocytosis by the alveolar macrophages [30]. The airway epithelium senses and

responds to inhaled antigens, leading to the regulation of innate and adaptive immunity through the release of inflammatory cytokines, chemokines and direct physical interactions with leukocytes that augment host defense mechanisms. Innate leukocytes and the airway epithelium are able to respond rapidly to danger signals through a conserved set of pattern-recognition receptors (PRRs), which are germ-line encoded receptors that recognize conserved pathogen-associated molecular patterns (PAMPs) or cell damage associated molecular patterns (DAMPs). The activation of the PRRs does not require an adaptive immune response. In addition to recognizing cell wall components, PRRs can also respond to an array of endogenous danger signals such as heat shock proteins, extracellular matrix components (*i.e.* hyaluronic acid), oxidized lipoproteins and complement factors released during tissue injury and cell death [31–33].

PRRs may be broadly categorized as trans-membrane receptors such as TLRs or cytosolic receptors, such as retinoic acid-inducible gene 1 (RIG-I)-like receptors (RLRs), and the NOD-like receptors or NLRs [31]. The TLRs recognize PAMPs such as components of microbial cell walls (TLR1, 2, 4 and 6), flagellin (TLR5), single stranded or double stranded viral RNA (TLR3, 7 and 8), or unmethylated DNA CpG motifs common to bacterial DNA (TLR9) [34]. Additionally, TLR10 is expressed in humans, although the specific ligand for it remains unknown. TLR3, 7, 8, and 9 are associated with endosomes and detect pathogen nucleic acids following endocytosis [35–39].

The TLRs play an essential role in early inflammatory responses to bacteria through the transcriptional upregulation of a variety of proinflammatory and antimicrobial peptides. A variety of endogenous molecular patterns released by injured cells as “danger signals” for tissue damage or DAMPs can also activate inflammatory responses *via* TLR-dependent signaling [40,41]. Allergens and viruses also activate the host immune response through the activation of the PRR system. PRRs within the airway epithelium recognize allergen-associated danger signals by protease dependent and protease independent pathways. Protease dependent effects include a direct cleavage of cell-surface molecules [42], or activation of G-protein coupled protease-activated receptors (PARs) [43–47]. The lectin family of cell-surface C-type lectins recognizes fungal cell wall components [48] including β -glucans that are responsible for some of the activation of AECs by allergens such as house dust mite (HDM) [49]. Activation of dectin receptors by β -glucans [49,50] that act as structural mimics of the adaptor protein MD2, leads to TLR4 signaling [51], activation of TLR2-dependant signaling [52] or induction of oxidative stress by intrinsic NADPH oxidase activity [53].

The mRNA expression of all ten Toll-like receptors has been reported in both human primary AECs and human AEC lines [54,55]; however, expression levels vary and TLR7 and 8 may not be expressed at functionally relevant levels [56]. Although TLR expression in alveolar epithelial cells has not been as well studied, primary human alveolar epithelial cells clearly express functional TLR2 and 4 [57,58], that protect against bacterial infection [59,60]. The expression of TLR3 and 5 are reported in the type II alveolar cell line (A549), derived from a human adenocarcinoma [61–64].

RLRs are RNA helicases expressed in the cytoplasm of cells that detect foreign RNA resulting from viral replication. Although both RIG-I and another RLR called melanoma

differentiation-associated protein 5 (MDA5) detect viral RNA and signal *via* the IPS-1 adapter protein to induce type I interferon (IFN) responses, only RIG-I is required for downstream gene induction following infection with influenza A and B, and human RSV [65]. In contrast, MDA5 but not RIG-I is required for IFN responses to live human RV-6 infection in human AECs [66]. Indeed, either RIG-I or MDA-5 is specifically required for maximal IFN response to a variety of other viruses as well [67]. RIG-I specifically recognizes 5'-triphosphate single stranded RNA and shorter double stranded RNA while MDA5 preferentially recognizes longer double stranded RNA [68]. The role of another RLR called LGP2 is less well defined. Unlike RIG-I and MDA5, LGP2 lacks a caspase recruitment and activation domain (CARD) required for interaction with the IPS-1 adapter protein and downstream signaling. However, cells lacking LGP2 have reduced IFN response to the vesicular stomatitis virus (VSV) and encephalomyocarditis virus (EMCV), suggesting that LGP2 cooperates with the other RLRs [69].

3. Respiratory infections as initiators of epithelial immune response

Infections with respiratory viruses (*e. g.* RV, RSV and influenza) and typical (*e. g.* *Streptococcus pneumoniae*, *Haemophilus influenzae*) and atypical (*e. g.* *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*) bacteria are major causes of morbidity and mortality globally [70,71]. These pathogens promote the induction and exacerbation of chronic respiratory diseases such as asthma and chronic obstructive pulmonary disease [72]. Humans with chronic respiratory diseases are also more susceptible to and suffer more severe consequences of infection [72,73]. Animal models that recapitulate the hallmark features of human disease have enabled the elucidation of the mechanisms that underpin these interactions [60,74–77]. Infection is typically initiated by exposure of the respiratory tract to viral particles or bacterial cells through inhalation of contaminated water droplets (Fig. 2). The infectious agents then bind to specific receptors on the surface of AECs. RVs bind to ICAM-1 (major group) or low density lipoproteins (LDLs, minor group), whereas influenza attaches to sialic acid residues [78]. This causes endocytosis of the viruses and once inside host epithelial cells they uncoat and initiate the viral replication process. Typical bacteria bind to a variety of cell surface receptors; for example, *S. pneumoniae* attaches to disaccharides (GlcNAc and GalAc), extracellular matrix and other receptors, while *H. influenzae* adheres to platelet-activating factor (PAF) receptors [79]. This enables these bacteria to colonize the cell surface, enter and grow or circulate through the epithelial cells, or pass deeper into tissues causing invasive disease. Atypical bacteria bind to mannose 6-phosphate (*C. pneumoniae*), and other as yet unknown receptors before internalization into vacuoles inside host cells [80,81] where the bacteria proliferate intracellularly until host cells are lysed enabling the infection of leukocytes and translocation around the body [82]. The interactions of these infectious agents with epithelial cells and their growth lead to the stimulation of innate immune receptors and responses and the production of IFNs and IFN stimulated genes [72,83]. These responses kill the invading pathogens and stimulate inflammation and adaptive immunity by the release of pro-inflammatory cytokines and

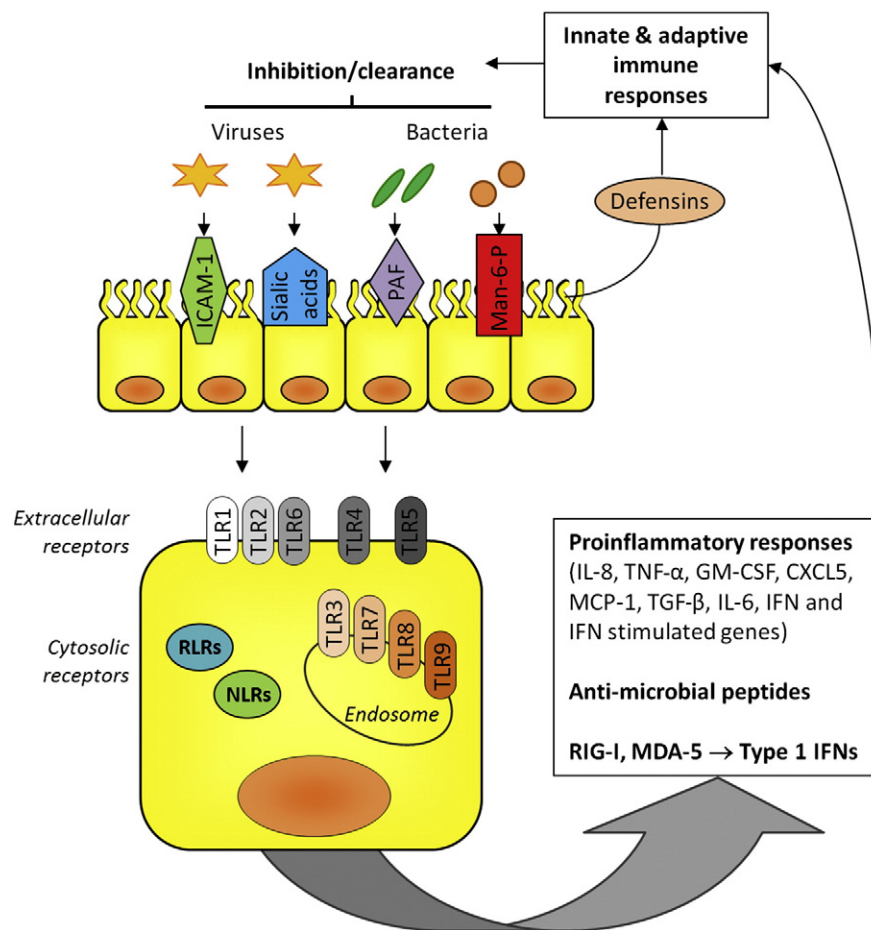


Figure 2 Cellular responses of the airway epithelium to infection. Viruses and bacteria bind to cellular receptors such as intercellular adhesion molecule 1 (ICAM-1) and sialic acid residues, and platelet-activating factor (PAF) and mannose-6-phosphate receptors, respectively. This enables the pathogens to internalize and/or replicate in airway epithelial cells. This induces the production of innate immune defensins and the stimulation of extracellular and intracellular innate immune receptors (TLRs, RLRs, NLRs). This leads to the generation of pro-inflammatory innate and adaptive immune responses and the production of interferons (IFNs). These events typically lead to the clearance of pathogens in healthy individuals. In patients with chronic respiratory diseases such as asthma and chronic obstructive pulmonary disease these defenses can be impaired leading to increased susceptibility to infection that drives inflammatory responses that in turn exacerbates the underlying disease.

drive DC activation. Subsequent humoral and DC-induced cell-mediated immunity results in the generation of B cells and CD4⁺ T helper and CD8⁺ cytotoxic T cells that further contain the infections. In addition the epithelium releases anti-inflammatory factors such as IL-10 that moderate pro-inflammatory responses. Thus, the respiratory epithelium is the primary controller of responses to pathogens, being both a primary defense site and the site of induction and control of the cascade of innate and adaptive responses. The adaptive responses that are induced are pathogen specific and are rapidly activated upon subsequent encounter more rapidly clearing the infection.

The presence of underlying inflammatory conditions alters immunity to pathogens that may undermine the protective effects of the epithelium. In asthma, high levels of IL-13 are present which suppresses pathogen clearance [84,85]. Asthmatics also have reduced IFN responses that could impair the innate immune response to infection [86,87]. In COPD increased inflammation is accompanied by increases in CD8⁺ cells that are

exhausted and may contribute to inflammation [77]. These deleterious responses may be reinforced by aberrant responses to infection that increase Th2 and inflammatory responses [88,89].

4. Function of the epithelium in innate immune responses and inflammation

The ability of alveolar epithelial cells to release chemotactic factors was recognized in a seminal paper published in 1990, describing the ability of A549 cells to release IL-8 in response to TNF or IL-1β [90]. The panel of cytokines released by alveolar epithelial cells has since expanded to include TNF [58], IL-6 [91], IFN [92], MCP-1 [93], TGF-β [94], GM-CSF [95] and CXCL5 [96] in response to a variety of stimuli including TNF, IL-1β, influenza virus, mycobacterium, Fas activation, ischemia-reperfusion and stretch. There have also been reports suggesting that type II cells release IL-1β in response

to LPS [97], but additional work is needed in this area. These studies collectively indicate that the alveolar epithelium is an important source of proinflammatory cytokines in the lung.

The relative importance of the lung epithelium as a source of proinflammatory cytokines and neutrophil chemoattractants relative to other sources such as macrophages has been addressed in several investigations. Transgenic mice expressing a mutant $I\kappa B-\alpha$ under the control of the surfactant protein C (SP-C) promoter that is expressed in the distal lung epithelium had impairment of the neutrophil response to LPS, associated with a decreased expression of the cytokines TNF, IL-1 β , MIP-2 and KC [98]. Since $I\kappa B-\alpha$ functions as a dominant negative inhibitor of NF- κB translocation, this impairment of NF- κB -dependent cytokine release was due to the inhibition of NF- κB in the lung epithelium. These findings were confirmed in another model with mice expressing a dominant negative $I\kappa B-\alpha$ under the control of the CC10 promoter that is expressed in Club (Clara) cells lining the entire airway in mice but only terminal bronchioles in humans [99]. Such a role of non-myeloid cells in innate immunity was further confirmed by experiments demonstrating that chimeric mice lacking the adaptor protein MyD88 in non-myeloid cells had impaired neutrophil recruitment in response to aerosolized *Pseudomonas aeruginosa* and reduced the control of bacterial growth [100]. These data suggest that TLR signaling via MyD88 in epithelial cells is required for early control of infection with *Pseudomonas*. The important role of the lung epithelium was further confirmed in a HDM model of allergen-induced inflammation that requires TLR4 expression on lung airway cells but not on myeloid cells [101]. Thus, TLR signaling in lung epithelium plays an important role in the inflammatory response to both bacterial pathogens and inhaled allergens.

In addition to releasing cytokines, the lung epithelium plays a role in both innate and adaptive immunity through release of antimicrobial peptides known as defensins. Defensins are small cationic peptides ranging in size from 3.5 to 5 kDa and are expressed in a wide range of tissues [102]. Their main functions are the direct inhibition of pathogens and their products and modulation of innate and adaptive immune responses [103]. There are two families of defensins: α -defensins and β -defensins. There are six different α -defensins; four are expressed in the azurophilic granules of neutrophils and are known as "human neutrophil defensins" (HNPs), whereas two are expressed in epithelia and are known as HD5 and HD6 [104]. The role of HD5 and HD6 in the lung is limited. Of the four human β -defensins, known as hBD1–4, all are expressed in the respiratory epithelium. hBD-1 is constitutively expressed [105], and hBD2–4 are induced in response to infectious or inflammatory stimuli [104]. Although the study of antimicrobial peptides has focused on the airway epithelium, it is clear that alveolar epithelial cells can also release defensins. For example, A549 cells release hBD2 and 3 following exposure to *Legionella pneumophila* [62,63] and hBD2 in response to LPS and IL-1 β [106,107]. Defensins can directly kill bacteria, viruses, fungi and protozoa; induce the aggregation of viruses or bacteria, and inactivate bacterial toxins. Defensins can also act as chemotactic agents for monocytes, macrophages, T-cells and DCs. Thus, defensins play an important role in the immune responses of the lung.

In summary, lung epithelial cells express most germ-line coded PRRs in the TLR and RIG-I-like receptor families. These

enable the early recognition of a wide variety of inhaled bacterial and viral pathogens as well as allergens such as the HDM antigen. In response to activation by PRRs, epithelial cells contribute to the initial immune response in the lung through the release of cytokines for leukocyte recruitment and activation and release of antimicrobial peptides. Impairment of epithelial activation pathways results in diminished inflammatory response and impaired control of infection. In contrast to the innate system, the adaptive memory immune response is complex and its nature depends upon the type and context of exposure since such exposures are often complex mixtures of allergens, particles and microbial products that activate multiple signaling pathways simultaneously.

5. Epithelial regulation of the adaptive immune response

The epithelium serves as a critical link in the host immune response both for homeostatic innate and adaptive immune responses, as well as the development of pathological inflammation. This latter paradigm is particularly true for the development of a Th2 skewed immune response that is associated with infiltration of the airway epithelium with critical effector cells such as MCs and eosinophils. Recent studies have clearly demonstrated that epithelial derived cytokines including IL-25, IL-33 and TSLP are upstream of classical Th2 cytokines such as IL-4, IL-5 and IL-13 that are involved in allergic inflammation. The most well established epithelial mechanism for the development of Th2 inflammation is the activation of DCs via the OX40 ligand and TSLP that enables DCs to prime CD4⁺ T cell towards a Th2 biased immune response [108]. More recently IL-25 and IL-33 have been shown to activate a newly identified population of lineage-negative lymphoid cells that produce IL-13 and also establish a Th2 biased immune response [109–112] and play a newly identified role in airway inflammation and AHR in response to viral infection [113]. Thus, in the appropriate context and genetic background, leukocytes amplify the effects of epithelial-derived IL-25, IL-33 and TSLP in response to environmental stimuli leading to chronic inflammation.

5.1. Epithelial regulation of IL-25, IL-33 and TSLP

The airway epithelium is equipped to secrete an array of innate immune molecules such as lysozyme, defensins, collectins and cytokines that provide protection against infection by bacteria, viruses and fungi [114]. Recent studies have identified the airway epithelium as a major source of IL-25, IL-33 and TSLP that regulates immune-mediated inflammatory airway diseases and the response to infections (Fig. 3). Among susceptibility factors for asthma, the genes for IL-33 and TSLP have emerged as two of the most important associations for the development of the disease [115].

There is considerable evidence implicating TSLP in the pathogenesis of inflammatory airway diseases. Substantially elevated levels of TSLP are found in airway biopsies of asthmatics [116] 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 [117,118]. Conversely, mice lacking the TSLP receptor (TSLPR) do not

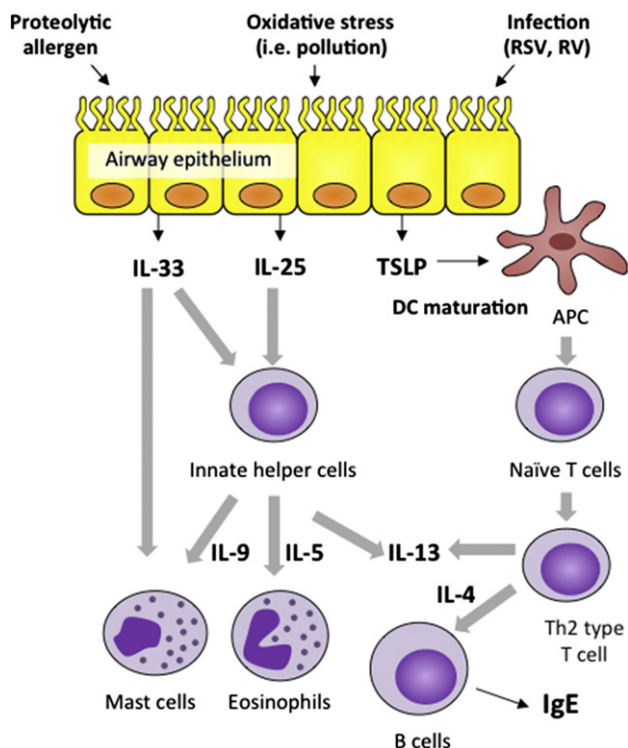


Figure 3 Regulation of innate and adaptive immunity through the airway epithelium. The airway epithelium responds to environmental exposures including proteolytic allergens, particles that cause oxidative stress, and infections with respiratory viruses with the generation of innate immune molecules as well as cytokines such as IL-33, IL-25 and TSLP that orchestrate the immune response. A population of innate helper cells is regulated by the epithelial production of IL-33 and IL-25. Mast cell and eosinophils are both regulated by IL-33 and TSLP. A key component of sensitization to inhaled allergens is the interaction between TSLP and antigen presenting cells such as DCs that present inhaled antigens to T and B cells leading to Th2 differentiation and IgE production within the appropriate context of co-stimulating signals.

develop eosinophilic airway inflammation following challenge with experimental allergen. Due to the potential importance of TSLP in allergic airway disease, the regulation of TSLP production by AECs has been investigated. Exposure of AECs to double-stranded RNA or infection with RV or respiratory syncytial virus (RSV) *in vitro* drives a marked upregulation of TSLP expression [114,119,120]. Further, exposure of AECs to diesel exhaust particles or RSV *in vitro* induces TSLP secretion [120–122]. These findings may help explain why respiratory viruses exacerbate allergic inflammation despite inducing Th1-type immune responses that could theoretically counter-balance Th2 mediated inflammation. Proteases also initiate the generation of reactive oxygen species (ROS) by AECs, leading to oxidation of lipids, signaling through TLR4, and the production of TSLP [123]. As many inhaled allergens exhibit endogenous protease activity, such proteolytic activity could contribute to the enhanced expression of TSLP by AEC. Following epithelial injury, knockdown of E-cadherin, or exposure to inflammatory cytokines (IL-1 β , TNF, IL-4 and IL-13) enhances the secretion of TSLP from AECs [114,124–126]. Recently, TSLP expression was linked to alterations in membrane proteins, such as caveolin-1

[25]. 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.

IL-33, a member of the IL-1 cytokine family serves as an alarmin and pro-allergic cytokine as well as a transcriptional regulator. IL-33 expression is increased in the airways of patients with severe asthma [127]. Further, IL-33 can induce TSLP production from epithelial cells [128]. IL-33 is produced in a fully active form by airway epithelial cells, fibroblasts, and smooth muscle cells and therefore might also act as link between cells in different tissue compartments to regulate the adaptive immune response. The regulation of IL-33 production by AECs is an active area of investigation. Recent studies in human subjects and mouse models of allergic airway disease indicate a central role of IL-33 in driving Th2-mediated inflammation, most likely through both the adaptive and innate immune systems. A novel population of IL-33-responsive innate immune cells, the type 2 innate helper cells, was found to produce hallmark Th2 cytokines, such as IL-5 and IL-13 [129]. Further study using fate mapping has revealed that innate helper cells are also an important source of IL-9 [130] that is important in the recruitment and retention of mast cells [131]. The relevance of innate helper cells to asthma is underscored by the identification of retinoic acid-related orphan receptor α (RORA) as an asthma gene in genome-wide association studies [132], as this gene encodes the transcription factor that is critical for innate lymphoid cell differentiation.

IL-25 belongs to the IL-17 cytokine family and is secreted by Th2 cells and mast cells (MCs). It was initially described as an amplifier of Th2 inflammation through the induction of other Th2 cytokines such as IL-4, IL-5 and IL-13 [133,134]. However, it is now clear that the direct delivery of IL-25 to the lungs results in AHR and eosinophilic pulmonary inflammation that develops *via* the IL-13 and signal transducer and activator of transcription (STAT)-6 pathway [135]. Blockade of IL-25 in an acute ovalbumin-driven model of allergic airway disease completely abrogated AHR [136]. Thus, a wider role for IL-25 in Th2 inflammation has been proposed. The combination of IL-25 with IL-33 can further promote the development of type 2 innate lymphoid cells that are critical in the early initiation of the Th2 response [110,112]. Biopsy studies of patients with asthma demonstrate elevated levels of IL-25 and its receptor IL-17RB transcripts compared with subjects without asthma [137] and elevated expression of IL-25 is inversely correlated with lung function [138]. Blockade of IL-25 in mice exposed to the HDM allergen or overexpressing SMAD 2 (which renders them more susceptible to airway remodeling and AHR following HDM exposure) completely suppressed the release of epithelial-derived innate cytokines and the classical Th2 IL-4, IL-5 and IL-13 cytokines [139]. Further, murine fibroblasts and airway smooth muscle cells express the IL-17RB and peribronchial collagen deposition was critically dependent upon IL-25 expression. As IL-25 has been shown to increase TSLP expression in human AECs [133] and murine AECs express IL-17RB [139] this suggests that IL-25 might also cooperate with TSLP and IL-33 to orchestrate airway remodeling and inflammation.

5.2. Recruitment and activation of DCs and lymphocytes

AECs can mediate and activate adaptive innate immune responses through interactions with DCs, T cells and B cells. AECs are capable of inducing DC migration to the epithelium *via* release of CCL20 [140], the only chemokine known to interact with CCR6 expressed by immature DC and Langerhans cells. CCL20 production by epithelial cells is induced by several stimuli, including the cytokines TNF, IL-1, IL-4, IL-13 and IL-17, PAMPs (dsRNA, PGN) and HDM allergens such as Der p1 [55,141–143]. The recruitment of DCs to the airways is essential for adaptive B, T and NK cell-mediated immune responses. TSLP strongly induces the expression of MHC class I and II co-stimulatory molecules such as CD40, CD80 and CD86 on DCs. Further, TSLP promotes DC-induced naive CD4⁺ T cell differentiation into Th2 cells that produce IL-4, IL-13 and TNF, but not IL-10 and IFN- γ . IL-33 enhances the production of IL-5 and IL-13 by Th2 cells but not by Th1 cells *in vitro*, and acts on murine bone marrow-derived DCs to induce a phenotype that is activated by IL-5 and IL-13, but not IL-4 or IFN- γ , production from naive CD4⁺ T cells [144].

5.3. Recruitment and activation of MCs and eosinophils

MCs are critical effector cells that are increased in the airways of patients with asthma [145,146]. MC products are released into the airways in response to allergen [147] and non-allergic triggers such as exercise [148]. MCs are also important in cigarette smoke-induced emphysema [77]. Recent studies have demonstrated that MCs are increased in the airway epithelium of patients with asthma, and that these MCs have a unique protease phenotype that is characterized by the high expression of the secretory granule proteases tryptase and carboxypeptidase A3 (CPA3), but low expression of chymase [149–151]. In rodents, mucosal MCs that express tryptase are an inducible population and are dependent upon T cells [152], and cytokines such as IL-3 and IL-4 [153] for their development, while connective tissue MCs are constitutive and express other granule proteins. The role of the epithelium in the retention and differentiation of these MC populations is a strong area of interest.

Epithelial cells generate stem cell factor (SCF) that is critical for the growth and differentiation of MCs [154–156]. The expression of SCF is increased in epithelial cells from the lower and upper airways of patients with asthma and rhinitis [154,155]. Epithelial cells support the growth of MCs *via* SCF, and the expression of chymase but not tryptase decreases in MCs co-cultured with epithelial cells [156]. In cultured epithelial cells, IL-13 induces the expression of SCF and conditioned media from epithelial cells treated with IL-13 cause a reduction in chymase expression [150].

The identification of the ST2 receptor for IL-33 and the TSLPR on mature MCs as well as CD34⁺ progenitor cells has further expanded the role of the interaction between MCs and the epithelium. It is well known that CD34⁺ cells with the capacity to give rise to MCs are increased in the peripheral blood [157] and airways [158] of patients with asthma. *In vitro* treatment of CD34⁺ cells with IL-33 accelerates their maturation into tryptase-containing MCs and the differentiation and

activation of human MCs are also influenced by IL-33 *via* the ST2 receptor *in vitro* [158]. In rodent models the role of IL-33 is less clear since ST2 deficient mice do not have an altered phenotype [159] when a sensitization model with an adjuvant that is not MC dependent is used [160,161]. However, models with short-term priming that are more MC dependent [162] demonstrate a reduction in AHR in ST2 deficient mice [163] suggesting a role for IL-33 in such models. The administration of IL-33 directly to the airways causes airway inflammation and AHR occurs in the absence of T and B cells [164] and the classic Th2 cytokine IL-4 [163]. The expression of CCR3 ligands is also induced by IL-33 in part through alternatively activated macrophages (AAM) [165]. The induction of airway inflammation and AHR by IL-33 involves the production of CCR3 ligands CCL11 and CCL24 by AAM [165] that may be involved in MC recruitment to the airways since CCR3 deficient mice have reduced MC recruitment to the airways, particularly intraepithelial MCs [166].

Epithelial derived TSLP may also play a role in MC differentiation in concert with IL-33. TSLP along with IL-33 accelerates the *in vitro* maturation of CD34⁺ cells and induces the secretion of Th2 cytokines and Th2-attracting chemokines [167]. TSLP also stimulates the production of high levels of Th2 cytokines by mature *in vitro* differentiated human MCs [124]. Further, CD34⁺ MC precursors are activated by epithelial cell supernatant in a TSLP-dependent manner [158].

Like MCs, intraepithelial eosinophils are increased in the airways of patients with asthma [168]. Airway infiltration with eosinophils is associated with more epithelial shedding and episodes of acute asthma [169]. There is a correlation between the number of mucosal eosinophils and lung function among patients with asthma [170]. Although the epithelium may serve as a target for eosinophil action, the epithelium itself may play a key role in the regulation and retention of eosinophils through the generation of chemokines and growth factors.

Airway eosinophils relative to circulating eosinophils have increased *ex vivo* survival and markers of activation [171–174]. The cytokines IL-3, IL-5, and GM-CSF are involved in the production, differentiation and survival of eosinophils [175–177], and recapitulate the distinct “primed” morphology of an airway eosinophil. This enhanced survival can be replicated *in vitro* using condition media from epithelial cells [178]. Epithelial cells produce GM-CSF [179] and the epithelial enhancement of eosinophil survival is at least partially dependent upon GM-CSF [180]. A recent murine study identified the airway epithelium as an important source of IL-5 that directly contributes to airway eosinophilia in this model [181].

Epithelial cells are also directly involved in the recruitment of eosinophils through the adhesion to epithelial intercellular adhesion molecule 1 (ICAM-1) [182]. Eosinophils transmigrate across polarized epithelial cells in tissue culture [183]. Although the mechanism is not fully understood, conditioned media from epithelial cells induce the chemotaxis of eosinophils *via* eotaxin and RANTES [24]. Eotaxin expression by epithelial cells is inducible by IL-1 β and TNF [184], and by IL-13 [185].

Recent studies have begun to delineate a direct role of IL-25, IL-33 and TSLP in eosinophil survival and activation. Peripheral blood eosinophils generate several chemokines and IL-6 in response to IL-25 [186]. In combination with IL-5, treatment of human eosinophils with IL-33 activates the production of several chemokines and increases the viability

of these cells [187–189]. TSLP activates both CD34⁺ progenitor cells and mature eosinophils [158]. There is significant *in vivo* evidence in model systems that IL-25, IL-33 and TSLP alter the number of eosinophils recruited to the airways as well as other features of asthma [118,133,165,190].

6. Pathological implications of epithelial immune activation

The inability of the airway epithelium of asthmatics to effectively defend the lung against normally innocuous inhaled agents suggests that the airway epithelial barrier of asthmatics is compromised. This idea is consistent with the hypothesis that barrier defects result in allergen leakage, mucosal immune activation, inflammation and ultimately clinical disease. Although a compromised barrier function is undoubtedly important in amplifying immune system activation, emerging data suggest that epithelial cell defects extend far beyond the physical barrier function.

The epithelium orchestrates and influences adaptive immune responses and functions as an interface between innate and adaptive immune regulation. An example is the interaction between epithelial cells and DCs through the release of cytokines and other soluble mediators and through direct cell–cell contact that may serve to play an important role in maintaining immune homeostasis. Increasing evidence indicates that both epithelial cells and DCs from asthmatic individuals exhibit distinct functional properties compared with healthy individuals as discussed previously.

One intracellular signaling pathway that is overexpressed in epithelial cells from asthmatics and plays a key role in viral infection is the mitogen activated protein kinase (MAPK) pathway, and in particular p38 MAPK [20,191]. Influenza virus relies on p38 MAPK to phosphorylate the viral nucleoprotein (NP) and facilitate ribonucleoprotein nuclear cytoplasmic shuttling that drives replication. Both H1N1 influenza and RSV infections are inhibited to similar degrees by the pharmacological inhibition of p38 MAPK, while pharmacological activation of p38 MAP kinase activation results in increased virus replication *in vitro* and *in vivo* [192,193].

Although the precise reason for upregulation of the MAPK cascade is not fully elucidated, the E3 ubiquitin ligase midline 1 (MID1) is increased in epithelial cells following HDM or RV exposure, and is correlated with CCL20 expression. In mice exposed to HDM, siRNA-mediated knockdown of MID1 significantly reduced AHR and allergic airway inflammation, expression of pro-inflammatory cytokines, NF κ B and p38 MAPK activity and the number of mucus-producing cells. The inhibition of MID1 also limited RV-induced exacerbation in this model of allergic airway disease [194].

The production of an effective immune response that limits the effects of inhaled environmental pathogens is a fundamental property of normal airway epithelium. Several studies have shown that epithelial cells isolated from asthmatics likely have a deficient innate antiviral immune response with respect to type I and III IFNs, in particular IFN- β , κ and λ release during RV infection [86,87,195], although this finding has not been universally accepted. Epithelial cells from asthmatics also undergo necrosis rather than apoptosis in response to IFN- β , resulting in greater viral loads, whereas supplemental IFN- β rescues the apoptosis response to RV and

viral loads are reduced [86]. While the mechanism underlying the blunted IFN response is incompletely understood, recent studies suggest that the increased production of TGF- β 2 may play a role. Exogenous TGF- β 2 increased RV replication and decreased IFN- β secretion in response to RV or dsRNA in human airway epithelial cells, while neutralizing antibodies to TGF- β 2 decreased RV replication and increased IFN- β expression in response to RV or dsRNA [196]. These findings may be relevant to human disease as endogenous TGF β 2 levels were higher in conditioned media from epithelial cell cultures obtained from asthmatic donors and the suppressive effect of TGF- β 2 antibodies on RV replication was significantly greater in these cells.

The pathological consequences of an aberrant epithelial immune barrier response may be responsible for many of the manifestations of common diseases such as asthma and COPD and are clearly critical in the host response to infection. For example, exposure of non-human primates to the HDM allergen with or without ozone demonstrated a remodeling of the airway in the form of reduced airway number, epithelial cell hyperplasia, increased mucus-secreting cells, AHR, interrupted postnatal basement membrane zone differentiation, reorganization of the airway vasculature, a modified epithelial distribution of nerves and Th2 skewing of the immune system. Intrinsic epithelial abnormalities noted in studies of epithelial cells isolated from subjects with airway diseases could drive many of these abnormalities [197]; such findings include mitotic dyssynchrony and defective clearance of apoptotic cells, mucus accumulation and exaggerated release of inflammatory mediators and growth factors [197,198].

7. Summary & conclusions

The epithelium of the lung, including the airways and alveolar space, is now recognized to play a central role in immune homeostasis. Through regulation of innate and adaptive immunity, the epithelium functions as a critical first defense against inhaled viral, fungal and bacterial pathogens. The dysregulation of this epithelial immune response is also strongly implicated in pathological states of inflammation such as asthma, acute lung injury and COPD. In this manuscript we report genetic, structural, and functional studies that support a primary role for the epithelium as a regulator of the lung immune response. Although the epithelium serves an essential physical barrier that may be altered in disease states, much recent work has identified a major role for the epithelium in the secretion of cytokines and antimicrobial peptides, and recruitment of leukocytes. The precise nature of the relationship between epithelial barrier dysfunction and the immune response as well as the deviation of the immune response towards pathological states of inflammation remains an active and fruitful area of investigation. A more complete understanding of how the epithelium regulates pulmonary immune function and airway homeostasis will provide new insights into the pathogenesis of asthma and other respiratory diseases as well as novel diagnostic and therapeutic opportunities.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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