Control of local immunity by airway epithelial cells

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The lung is ventilated by thousand liters of air per day. Inevitably, the respiratory system comes into contact with airborne microbial compounds, most of them harmless contaminants. Airway epithelial cells are known to have innate sensor functions, thus being able to detect microbial danger. To avoid chronic inflammation, the pulmonary system has developed specific means to control local immune responses. Even though airway epithelial cells can act as proinflammatory promoters, we propose that under homeostatic conditions airway epithelial cells are important modulators of immune responses in the lung. In this review, we discuss epithelial cell regulatory functions that control reactivity of professional immune cells within the microenvironment of the airways and how these mechanisms are altered in pulmonary diseases. Regulation by epithelial cells can be divided into two mechanisms: (1) mediators regulate epithelial cells' innate sensitivity in *cis* and (2) factors are produced that limit reactivity of immune cells in *trans*.

INTRODUCTION

Airway epithelial cells: beyond the barrier function

The simplified equation of cellular or aerobic respiration is $C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$. The vital consequence of this equation is that multicellular organisms, such as mammals, need a specialized organ to facilitate gas exchange between blood and air. In mammals, this is achieved by the lung, an organ of the respiratory system. The latter can be divided into the conducting airways (consisting of the upper respiratory tract, trachea, and bronchi) and the respiratory part (consisting mainly of alveoli). The lung must be actively ventilated to satisfy the amount of air needed. Therefore, 10–20,000 l of air per day is ventilated over the roughly 100 m^2 surface of the lung. Thus, the lung is by far the largest organ of the human body with direct contact to the atmospheric environment, and, therefore, is prone to infectious attack. Moreover, to facilitate effective gas exchange, the membrane separating blood from inhaled air is only 1–2 μm thick. Thus, the interior of the human body is separated from the environment by a tennis court-sized surface, which has a thickness of roughly 1/10th the diameter of the cell nucleus. This makes the lung an attractive entry gate for pathogens. To prevent infection, the lung is equipped with several defense mechanisms; specialized cells of the immune system including alveolar macrophages and dendritic cells are able to sense and kill pathogens and to mount a protective inflammatory response.[1]

For decades, the perception of epithelial cell function has mainly been to build up a physical barrier to limit entry and to foster removal of pathogens. However, it has become clear that airway epithelial cells have a much more active role in the initiation of immune reactions. Besides the expression of pattern recognition receptors (PRRs) on professional immune cells (alveolar macrophages, sub- or intraepithelial dendritic cells), airway epithelial cells have also been shown to express Toll-like receptors,[2] RIG-I,[3] C-type lectins,[4,5] and inflammasome components.[6–8] So far, the relevance of the recently discovered cyclic GMP-AMP synthase in respiratory epithelial cells[9] remains unclear. The specific PRR/pathogen-associated molecular pattern (PAMP) combinations relevant for detection of certain pathogens have been comprehensively reviewed previously.[10] Thus, epithelial cells are equipped to participate in innate detection of microbial encounter.

Epithelial cells induce an airway-specific immune microenvironment

Air does not only contain potential pathogens but is also contaminated with organic or inorganic material, e.g., microbial cell wall constituents such as lipopolysaccharide (LPS). These substances will be sensed by PRRs expressed by airway epithelial cells, and have been demonstrated to be sufficient to induce an inflammatory response if systemically applied.[11,12] Obviously, if the same were true for the respiratory system this

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would lead to frequent, if not chronic, inflammation of the fragile respiratory tissue towards harmless, low-dose microbial pollutants. As inflammation inevitably induces some degree of collateral damage, this would eventually lead to a destruction of the fragile pulmonary system.

We therefore propose that *mechanisms in the respiratory system have evolved to keep immune functions under control.* This hypothesis follows the concept of specific immune microenvironments that shape and adjust local immune responses to organ-specific needs.[13] Obviously, microenvironment-specific cells must exert those regulatory effects on professional immune cells. Thus, airway epithelial cells, the first cells to make contact with inhaled microbial products or antigens, not only behave as innate immune *sensors* but also as *immune modulators* in parallel. The modulatory function is of specific importance *during homeostasis*, whereas the sensor function is needed in case of true microbial threat. Indeed, airway epithelial cells have been demonstrated to control local innate and adaptive immune responses, thereby keeping potentially damaging immune responses under control. We suggest that under homeostatic conditions, an important function of airway epithelial cells is to modulate the sensitivity of innate immune sensing. Various regulatory cascades and mediators have been shown to be involved in this process. Upon challenge with antigens or pathogens exceeding a certain threshold of tolerance, this inhibitory microenvironment might switch to a stimulatory one that now contributes to the initiation of immune responses. A logical consequence of such a concept is that spontaneous loss of these modulatory factors might be involved in the development of chronic airway diseases. Specific factors involved in this regulatory network and how these factors are modulated under various diseased conditions are the matter of this review. We will mostly focus on airway epithelial cells, but some of the regulatory mechanisms have also been shown to occur at the alveolar level. Whether differences in regulatory circuits based on the anatomical hierarchy of epithelial cells in the lung exist is not known in detail.

Under homeostatic conditions, control of professional immune cells' reactivity in the microenvironment of the airways can mechanistically be divided into two strategies. On the one hand, epithelial cell-derived mediators or intrinsic properties regulate sensitivity and reactivity of lower respiratory tract epithelial cells themselves (autocrine or *cis*-acting factors). On the other hand, epithelial cells produce factors that act on local immune cells including dendritic cells, macrophages, and lymphocytes (paracrine or *trans*-acting factors) (**Figure 1**).

CIS-ACTING FACTORS REGULATING AIRWAY EPITHELIAL CELLS' SENSING FUNCTION

Supported by various experimental observations, we suggest that epithelial cells under homeostatic conditions are hyporesponsive towards microbial stimulation, thereby adjusting the "reaction" threshold to the local microbial burden. The default setting of airway epithelial cells is "hyporesponsive" towards proinflammatory products, and this can be achieved by regulating epithelial-intrinsic mechanisms.

Organization of the epithelial barrier

The epithelium of the lower respiratory tract is built up by several different cell types, varying in the composition from proximal to distal. The main airway cell types forming the pseudostratified epithelium in the conducting airways are ciliated epithelial cells, non-ciliated mucous goblet cells, club cells (originally known as clara cells), and undifferentiated basal cells. The alveolar epithelium consists only of type I and type II alveolar cells. The mucus layer and the physical barrier formed by the epithelium contribute to a first line of defense. In consequence, epithelial cells are physically separated from potential stimulatory factors by mucus, a mechanism that also clears the lung from potentially activating compounds (mucociliary clearance (MCC) system), or surfactant, which gets eventually cleared by phagocytosis of alveolar macrophages.[14,15] Moreover, the physical barrier produced by the epithelium itself separates potential proinflammatory signals from (reactive) interstitial macrophages, dendritic cells, and lymphocytes. Originally, this protective barrier has been shown in the intestine for the gut microbiota. As this effect is mediated by the secretion of anti-microbial peptides and the airway epithelium is also able to produce similar factors, a protective barrier can be expected to be operative in the lung.[16–18]

Epithelial cells separate microbial products from the body's interior, thus limiting microbial access to immune sensors

An important function[19] of epithelial cells is to act as a physical barrier towards the outer environment. Transcellular junctions are formed by apical tight junctions, adherens junctions, and desmosomes. Tight intercellular connections are important not only to prevent paracellular transport of ions, water, or cells into the airway lumen but also to physically separate microbial compounds from professional immune cells. Tight junctions limit access of airborne substances to the body interior. Professional luminal antigen sampling thus requires specific mechanisms, and indeed, it has been demonstrated that $CD103^+$ intraepithelial dendritic cells are able to stretch cellular extensions into the airway lumen, bypassing this barrier.[20–22] Even though alveolar macrophages are found at the luminal side in the alveolar space, these cells do not get direct access to pathogens because of a layer of surfactant. Moreover, they reside in the most distal part of the lung system and are therefore situated behind the filtration barrier of the upper or conducting airways. The presence of macrophages in the lumen also results in a spatial segregation allowing localized, restricted defense reactions without directly inducing a system-wide response. Additionally, cells sampling luminal content are educated by various paracrine-acting factors derived from epithelial cells further discussed below.

The importance of a functional tight epithelial barrier is demonstrated in the context of acute respiratory distress syndrome. Even though this disease is primarily driven by a loss of endothelial barrier integrity, full-blown disease develops only

if the epithelial barrier is impaired in parallel.[23,24] Loss of epithelial cell integrity allows neutrophils to get in contact with luminal bacterial products. This leads to the release of reactive oxygen species and other factors (e.g., neutrophilic elastase), which further exacerbate the disease. Moreover, several studies demonstrated impaired epithelial barrier function in asthmatic patients, and increased shedding of epithelial cells, impairing the barrier function, has also been suggested.[25,26]

Mucus as a defense layer limiting access to antigens and microbes

To strengthen the physical barrier, goblet cells are interspersed in the airway epithelial layer or in the submucosal glands. They secrete mucins into the airway lumen to build up a protective layer of mucus. Mucins are high-molecular-weight glycoproteins, of which at least 16 have been identified to be expressed in the lung (MUC1, MUC2, MUC4, MUC5AC, MUC5B, MUC7, MUC8, MUC11, MUC13, MUC15, MUC16, MUC18, MUC19, MUC20, MUC21, and MUC22).[27] Interestingly, not only goblet cells produce mucins but also club cells (MUC5AC)[28] and alveolar epithelial cells (MUC1).[29] Mucins are heavily post-translationally modified and extremely hydrophilic. Their main function is to form a protective mucus barrier to trap inhaled particles, which are subsequently actively transported out of the respiratory tract by MCC. Moreover, mucins also display anti-microbial, antiprotease, and antioxidant activities,[27] thereby contributing to unspecific, innate immune defense. Failure in the MCC leads to the accumulation of mucus in the lung, resulting in chronic airway inflammation, as seen in patients with chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), or primary ciliary dyskinesia.[30] Coordinated beating of cilia requires intense cell–cell communication via connexins, exemplifying the importance of an intact epithelial cell layer. Seven airway mucins (MUC2, MUC5AC, MUC5B, MUC6, MUC7, MUC8, and MUC19) are considered to be secreted into the airway lumen, whereas the others remain membrane-tethered on the apical side of the epithelium.[27] The gel-like mucus, built by the secreted mucins, floats atop an apical periciliary liquid layer, also known as airway surface layer. The properties of this layer are important for effective MCC and are determined, on the one hand, by ions and other small molecules secreted, on the other hand, by epithelial cells, but most importantly by membrane-tethered mucins, especially MUC1, MUC4, and MUC16.[31] MUC5AC and MUC5B are the most abundantly secreted mucins in the conducting airways. Even though the exact role of these two mucins in regulating the airway immune system is unknown, it has been reported that MUC5AC is inducible under inflammatory condition, while MUC5B is constitutively expressed.[32,33] The relative importance of MUC5B compared with MUC5AC in homeostatic non-diseased conditions is reflected by the phenotypes of the respective knock-out mice. Muc5ac-deficient mice are viable and do not display any respiratory disease symptoms. However, these mice fail to recruit neutrophils in response to an acute challenge with *Trichuris muris* or in acute lung injury.[34,35] In contrast, Muc5b-deficient mice spontaneously develop severe pulmonary pathology, mucus obstruction, and chronic bacterial infection.[36] Most likely, this is due to the inability to clear particles or pathogens along with a decrease in interleukin-23 (IL-23) production by macrophages or dendritic cells.[36] Mucus production and mucus transport, therefore, limit contact between airway epithelial cells and immunostimulatory substances. This is especially true for particles having an aerodynamic diameter larger than 2–3 μm, which are prone to precipitate after inhalation in the conducting airways.[37]

An important immunomodulatory mucin is MUC1, expressed not only in goblet cells but also in lymphocytes and dendritic cells. After secretion, it remains tethered to the cell membrane, where it can be released by neutrophilic elastase. Exemplifying its modulatory function, it has been shown that Muc1 is a receptor for *Pseudomonas aeruginosa*, an opportunistic lung pathogen. Muc1-deficient mice displayed better lung clearance of *P. aeruginosa* compared with wild-type littermate.[38] This was accompanied by increased tumor necrosis factor-α (TNFα) and KC levels in the bronchoalveolar lavage liquid, resulting in elevated numbers of neutrophils and clearance of *P. aeruginosa*. As *P. aeruginosa* is mainly recognized by epithelial Toll-like receptor 5 (TLR5)/flagellin, this observation was interpreted as an indication that Muc1 has an inhibitory function on epithelial TLR5 signaling.[39]

Epithelial cell-intrinsic microbial hyporesponsiveness

An increasing number of reports now indicate that TLR signaling is actively suppressed in the airway and also in alveolar epithelial cells under homeostatic, non-stressed conditions. This hyporesponsiveness of airway cells has been observed for TLR2 and TLR4 agonists.[2,40–42] Even though these cells express TLR2 and TLR4, direct stimulation with Gram-positive bacteria or LPS does not result in a strong induction of proinflammatory cytokines. It has been postulated that the lack of expression of CD36, a cofactor of TLR2, as well as MD2 and CD14, cofactors of TLR4, are responsible for this hyporesponsiveness.[2,41,42] Regulation of coreceptors might be an organ-specific mean to adjust the sensor threshold (of various innate immune receptors) to the specific needs of a given compartment.

Similar observations have been made for alveolar epithelial cells. Even though they express TLR2 and TLR4, alveolar type II (AT-II) cells in contrast to alveolar type I (AT-I) cells seem to be hyporesponsive towards LTA and LPS and act anti-inflammatory.[43] This tolerance is partially mediated by an epigenetic mechanism induced by repeated challenge of these cells with LPS or LTA[44] (reflecting a phenomenon known as endotoxin tolerance). In addition, surfactant produced by AT-II cells can also have anti-inflammatory properties. Especially SP-A and SP-D, two of four surfactant proteins (SP), and phosphatidyl glycerol-containing surfactant vesicles are able to bind directly to TLR2, TLR4, MD2, and CD14, thereby blocking the interaction of PAMPs with these receptors.[45–48] Similarly, SP-C has also been shown to bind

LPS, thereby scavenging potential harmful LPS in the alveoli. Interestingly, LPS and other proinflammatory cytokines are able to suppress SP expression in a dose-dependent manner in AT-II cells.[49] This indicates that the initial levels of SP present in the alveoli dictate a threshold for sensing LPS, which must be overcome to reduce the anti-inflammatory properties of SP on alveolar macrophages and AT-I cells. Once this threshold is exceeded, LPS and proinflammatory cytokines are able to suppress further SP expression and potentiate inflammation[49] (feedforward amplification). The threshold needed to overcome this inhibition can be achieved not only by a single bolus dose but could also accumulate over time because of disturbances in the clearance of the lung.[36]

Epithelial tight junctions result in polarization of airway cells. Restriction of sensor molecules to the inaccessible basolateral side has been suggested as another mechanism contributing to reduce PRR sensing of surface-borne stimulation. Thus, TLR4 is less expressed at the apical side.[40,50] Only virulent pathogens, overcoming the physical barrier, could access the sensor molecules, indicating true and present danger within this compartment.[40] Of note, different localization of TLR5 dependent on the anatomical localization (basolateral in bronchi, but apical in alveolar cells) has been observed and seems to parallel the burden of microbial contact.[51]

TRANS-ACTING FACTORS

Besides the regulation of airway epithelial cells' intrinsic sensitivity towards microbial sensing, pulmonary epithelial cells produce a variety of factors that regulate professional immune cells present in the respiratory microenvironment. Under homeostatic conditions, these cells mainly encompass macrophages, dendritic cells, and lymphocytes. Such factors are found in three functional groups: effects by direct cell–cell contact, local acting cytokines, and lipophilic factors (**Figure 1**).

Contact-dependent regulation of airway and lung immune reactions

The reactivity of alveolar AT-I cells towards LPS is markedly increased in the presence of alveolar macrophages.[43] This effect is most likely mediated by direct cell–cell contacts as conditioned media of alveolar macrophages showed the opposite effect. Interestingly, the presence of AT-II cells decreased TNFα and IL-6 production of alveolar macrophages upon LPS challenge. This effect could not be seen in mice deficient in CD200.[52] CD200 is the ligand for CD200R, which is expressed in all myeloid cells. However, the highest levels are found in alveolar macrophages.[52,53] CD200-deficient mice or CD200R agonists were able to increase or ameliorate, respectively, influenza-induced pulmonary inflammation,[52] thus proving an immunosuppressing function of AT-II cells

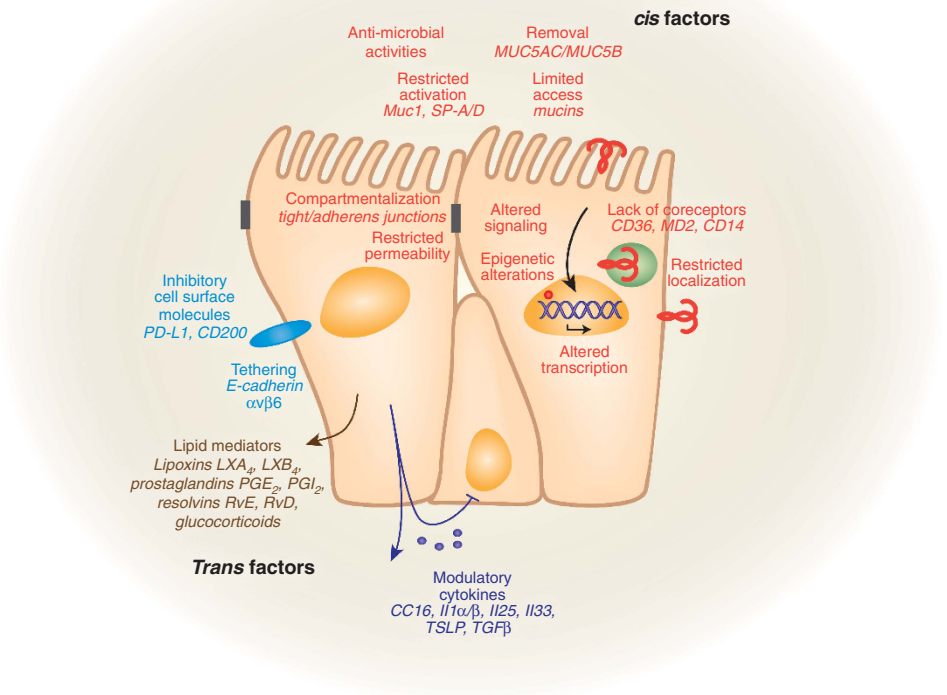

Figure 1 *Cis*- and *trans*-acting immune-modulatory factors of airway epithelial cells. Airway epithelial cells limit their sensory potential under homeostatic conditions by *cis*-acting factors that increase the threshold of activation by luminal microbial stimuli. In addition, airway epithelial cells act on professional cells, inducing a specific microenvironment in *trans*, by expressing inhibitory cell surface molecules and secretion of modulatory cytokines and lipophilic factors.

expressing CD200- on CD200R-expressing alveolar macrophages. Moreover, CD200R deficiency protected mice from influenza-induced secondary bacterial superinfection, as influenza is able to induce CD200 expression in the lung.[54] The anti-inflammatory properties of CD200 have also been addressed for therapeutic usage. It was shown in a rat model of asthma that reduction of infiltrating Th2 lymphocytes and inflammatory dendritic cells could be achieved by intratracheal administration of recombinant CD200.[55] CD200 is not only expressed by AT-II cells but also in Club cells extending the previous regulatory circuit to the alveolar duct and ciliated bronchiolar epithelium with apically localized CD200.[56] As myeloid cells can only be found in the subepithelial compartment in the conducting airway, this regulation might be especially important under inflammatory conditions when evaded leukocytes can be observed in the airway lumen.

Another immunomodulatory cell surface protein expressed by pulmonary epithelial cells is PD-L1.[57–59] PD-L1 is known to be expressed by antigen-presenting cells and to mediate T-cell apoptosis, anergy, and functional exhaustion by activating its receptor PD-1.[60] The functional relevance of a PD-L1/PD-1 interaction between airway epithelial cells and T cells has been demonstrated in the context of human metapneumovirus. This ligand–receptor pair mediated functional impairment of $CD8^+$ T cells[57,61] and increased clearance of influenza.[58] PD-1 expression has also been shown on dendritic cells[62] or macrophages[61,63] and adoptively transferred PD-1-deficient DCs into wild-type mice performed better in clearing bacterial pathogens from the blood stream as compared with wild-type DCs. This effect was independent of the presence of T and B cells, arguing for a regulatory function in the innate immune system as well.[62] This observation also implies a potential interaction between airway epithelial cells and myeloid cells. Indeed, it was shown recently that airway epithelial cells are able to dampen LPS-induced TNFα release in monocytes and macrophages partly dependent on epithelial PD-L1.[59] In addition, E-cadherin expressed in all airway epithelial cells is able to modulate $CD103^+$ leukocytes, namely intraepithelial dendritic cells and T cells.[64–66] $CD103^+$ dendritic cells have been proposed to be important for the induction of T_{reg} cells in the intestine; however, a more proinflammatory phenotype of these cells have been described in the lung.[67–69] However, CD103-deficient mice performed less well in a murine model of asthma.[64]

Epithelial cell-derived modulatory cytokines

Besides cell–cell contacts, immunomodulatory activities have also been identified in supernatants of epithelial cells. Moreover, molecules mediating cell–matrix interactions such as $\alpha_V\beta_6$-integrin have immunomodulatory properties. Integrin $\alpha_V\beta_6$ binds to the extracellular matrix proteins fibronectin, vitronectin, and tenascin, and the expression is restricted to epithelial cells of the endometrium, lung, and kidney. However, unlike other integrins, expression of integrin $\alpha_V\beta_6$ can also be induced upon injury or inflammation in various organs.[70–73] It was shown that mice deficient in integrin β_6 developed airway hyperresponsiveness to acetylcholine, a symptom associated with asthma, had increased infiltration of lymphocytes into the conducting airways,[74] and displayed age-related emphysema.[75] Subsequently, it could be shown that epithelial integrin $\alpha_V\beta_6$ is able to bind and activate latency-associated peptide/tumor growth factor-β (TGFβ) complexes, thereby locally regulating TGFβ activity and function.[76] Mice lacking integrin $\alpha_V\beta_6$ were protected against OVA-induced allergic asthma despite increased basal inflammation as TGFβ activates mast cells.[77] Moreover, acute lung injury and pulmonary fibrosis was reduced, as TGFβ is able to modulate epithelial cell permeability[78] and to induce myofibroblast development, respectively.[76,79] The local activation of TGFβ by integrin $\alpha_V\beta_6$ leads to a high level of TGFβ in the lung[80] and it has been demonstrated that TGFβ inhibits Th1/Th2 differentiation. In line with these results, airway epithelial cell-derived TGFβ also blocked lymphocyte proliferation.[81] The context-dependent functions of TGFβ confirm the central immunomodulatory function of epithelial cells as its local activation is performed by integrin $\alpha_V\beta_6$ expressed by epithelial cells.

Another anti-inflammatory and immunomodulatory cytokine with a specific function in the lung is CC16, also known as CCSP or uteroglobulin, and is produced exclusively by club cells. For a long time, serum CC16 levels have been proposed as a prognostic biomarker for COPD.[82] Initial attempts failed to demonstrate physiological significance of CC16 in cigarette-induced bronchitis and emphysema using CC16-deficient mice.[83] However, a recent study showed increased susceptibility of CC16-deficient mice to emphysema and peribronchial fibrosis using a long-term cigarette smoke exposing protocol.[84] CC16 was able to inhibit LPS- or IL-1β-mediated nuclear factor-κB activation in airway epithelial cells[85–87] and LPS- or IL-13-mediated mucus hypersecretion.[87] In addition, cigarette smoke induction of IL-8 release was inhibited[88] and CC16 modulated protein expression of macrophages. However, the significance of this observation is limited.

Airway epithelial cells shape immune response by cytokine secretion

Besides these anti-inflammatory cytokines, pulmonary epithelial cells are also able to produce proinflammatory or immunomodulatory cytokines to shape a proper immune response. Most of the research carried out so far focused on the development of asthma and mounting of a Th2 skewed inflammation under conditions of immune challenge.[89,90]

In this respect IL-25, IL-33, and TSLP have been heavily investigated and several recent reviews have been discussing the role of these cytokines in detail.[89,90] IL-25 and TSLP have especially gained interest as potential therapeutic targets, as both cytokines have been associated with asthma in genome-wide association studies.[91,92] All these cytokines are important in recruiting ILC2 cells, which subsequently produce high levels of cytokines (mainly IL-4, IL-13, IL-5) and mediate a Th2-type inflammation. This leads to a promotion of allergic pulmonary inflammation. IL-25, IL-33, and TSLP have also been discussed

to mediate *in situ* hematopoiesis, thereby regulating inflammatory Th2 responses.[93] They were able to induce recruitment of hematopoietic stem/progenitor cells to mucosal surfaces upon inflammation.[93–97] Of note, differentiating ILCs from hematopoetic stem/progenitor cell is difficult as they share similar cytokine profiles and similar surface markers such as CD90 and CD117.[98] Therefore, the role of ILC and *in situ* hematopoiesis in the literature might be misleading and care must be taken in future research to differentiate both mechanisms under pathologic conditions.

IL-33 belongs to the IL-1 superfamily that comprises of IL-1α/β, IL1-Ra, IL-18, IL-33, IL-36α/β/γ, IL-37, and IL-36Ra and IL-38. IL-1β is a well-known cytokine involved in several inflammatory diseases. IL-1β is regulated at the transcriptional level as well as post-translationally, as the biologically inactive pro-IL-β must be activated by the inflammasome. It has been demonstrated that sputum cells from asthmatic patients display lower IL-1β secretion upon LPS stimulation compared with healthy control,[99,100] and therefore a role of IL-1β in asthma development has been postulated.[101] However, contradictory observations for mice deficient in IL-1β or deficient in enzymes involved in activation of IL-1β (e.g., caspase-1, Asc, Nlrp3) in murine models of asthma have been described.[102–105] Mice deficient in IL-1R, the receptor for IL-1α/β, have been demonstrated to be protected from house dust mite-induced asthma[106] and neutrophilic inflammation in a murine model of CF.[107] Interestingly, both studies suggest a more important role of IL-1α than IL-1β in these pathologies. Taken together, the ability of epithelial cells to secrete cytokines under inflammatory conditions to shape the immune response in a lung-appropriate manner further demonstrates the importance of these cells once hyporesponsiveness is disturbed.

Lipophilic factors regulate homeostatic lung immune reactivity

Lipoxins. Derivatives of the arachidonic acid (AA), a 20-carbon polyunsaturated fatty acid, including lipoxins (LXs) and prostaglandins (PGs) contribute to lung physiology. Both groups of lipids, also known as eicosanoids, are synthesized by airway epithelial cells and exert their effect on various immune cells. During recent years interactions between different immune cells and airway epithelial cells involving these factors have been shown. LXs are a good example of this crosstalk as the synthesis of LX depends on various cell populations in a kind of transcellular production. LXs (LXA_4, LXB_4) are anti-inflammatory derivatives of AA and are produced by two enzymes, 15-lipoxygenase, expressed in airway epithelial cells and macrophages, and 5-LO, expressed in eosinophils, neutrophils, monocytes, and macrophages.[108] Epithelial 15-LO converts AA into 15-hydroxyeicosatetraenoic acid, which is further processed by 5-LO into LXA_4 and LXB_4. It has been shown that human airway epithelial cells convert AA predominantly into 15-hydroxyeicosatetraenoic acid and to a much lesser extent into PGs, leukotrienes, or others.[109] The transcellular synthesis of LX was initially described to occur in the interplay of alveolar macrophages and neutrophils,[110] but has been subsequently demonstrated for epithelial cells and neutrophils as well.[111] The principal receptor for LXA_4 is ALX/FRP2,[112] but LXA_4 can also trigger other receptors; the specific receptor for LXB_4 has not been identified so far. LXs act generally as a "stop signal" during inflammatory reactions[108] by inhibiting neutrophilic chemotaxis, modulating contraction of pulmonary arteries, and acting as antiphlogistics. LXs are also able to inhibit nuclear factor-κB signaling, thereby inhibiting LPS-mediated proinflammatory cytokine secretion by neutrophils and macrophages.[113] Moreover, LXA_4 modulates transepithelial ion transport and leads to increased airway surface liquid heights.[114] This is of special importance in the context of CF, where the mutation of cystic fibrosis transmembrane conductance regulator leads to a decrease in airway surface layer height. Moreover, transepithelial ion transport is able to modulate the anti-bacterial effect of the airway mucus.[115] In addition, LXA_4 is able to modulate the repair of the airway epithelium by regulating the proliferation and migration of epithelial cells.[116]

Prostaglandins. AA can also be metabolized by cyclooxygenase (COX) into PG H_2 (PGH_2). PGH_2 can then be converted into the prostanoids PGD_2, PGE_2, PGF_2, and PGI_2 by their specific synthases, namely PGDS, PGES, PGFS, and PGIS. Two different COX enzymes (COX1, COX2) have been identified so far and are the principal targets for nonsteroidal anti-inflammatory drugs. COX1 is considered to be constitutively expressed and important for homeostatic PG synthesis. COX2 expression can be induced under inflammatory conditions by cytokines, such as IL-1, IL-2, and LPS.[117] However, COX2 seems to be constitutively expressed in human and murine lung epithelial cells.[118,119] In principle, all prostanoids are detectable in lung but only PGE_2 and PGI_2 are synthesized by airway epithelial cells.[109,120,121] PGE_2 is the most abundant PG produced by epithelial cells, both airway and alveolar, under homeostatic conditions.[109,121] Even though PGE_2 has been described as a proinflammatory mediator, the importance of PGE_2 under homeostatic conditions is to suppress inflammatory processes in the lung.[122] In line with this inhibitory function, PGE_2 concentrations in sputa of asthmatic patients are negatively correlated with the number of infiltrating eosinophils[123,124] and inhaled PGE_2 is beneficial for asthmatic patients.[125–128] Moreover, epithelial cell-derived PGE_2 dampens immunoreactivity of pulmonary dendritic cells by suppressing LPS-induced IL-12 and TNFα secretion and increasing IL-10 secretion in parallel.[119,129] PGE_2 is also able to regulate gap junction communication of airway epithelial cells, and thereby airway surface layer volume.[130]

An important role of alveolar epithelial-derived PGE_2 has been demonstrated in the context of pulmonary fibrosis,[131] as decreased PGE_2 levels were detectable in pulmonary fibrotic patients.[132,133] In this disease, PGE_2 seems to counteract TGFβ to suppress the activation of fibroblasts into myofibroblasts. Effects of PGE_2 are mediated by four G-protein-coupled EP receptors (EP1–4). Most of the immunomodulatory and anti-inflammatory potential of PGE_2 is mediated through EP2 and EP4 receptors.[119,120] EP2-deficient mice displayed stronger

bronchoconstriction in several models of allergic airway disease, whereas EP4 mediated the repression of LPS-induced cytokine secretion.[119]

In contrast to the homeostatic levels of PGE_2, the levels of PGI_2 are markedly increased under inflammatory conditions when PGI_2 becomes the major epithelial cell-derived COX product in the lung. PGI_2 is produced by PGI synthase and signals through its G-protein-coupled receptor IP (prostacyclin receptor).[120] Despite the well-known function of PGI_2 in the cardiovascular system, it is also able to suppress proinflammatory cytokine secretion and to increase IL-10 production by bone marrow-derived dendritic cells.[134] In line with this anti-inflammatory potential, IP-deficient mice performed less well in bleomycin-induced pulmonary fibrosis.

Resolvins. Resolvins are generated from the ω-3-polyunsaturated fatty acids, eicosapentaenoic acid, giving rise to the E-series of resolvins (RvE1/2), and docosahexanoic acid, giving rise to the D-series of resolvins (RvD1–4). Airway epithelial cells from asthma and CF patients have depleted intracellular stores of docosahexanoic acid, which implies dysregulated RvD synthesis.[135] Similar to LXs, resolvins require a multistep transcellular biosynthesis and are considered to mediate the resolution of inflammatory processes.[136] The production of RvE1/2 depends on epithelial COX2, which gets acetylated by aspirin, whereas RvD is produced by epithelial 15-LOX and neutrophilic 5-LOX.[137] RvE1 h0as been shown to promote clearance of bacteria in a murine model of pneumonia. Administration of RvE1 protected mice from experimental allergic asthma and RvD1 promoted resolution in a murine model of acute lung injury.[138] A potential molecular mechanism for these observations is Resolvin-mediated nuclear factor-κB inhibition and activation of phosphoinositide 3-kinase or extracellular signal-regulated kinase.

Local production of glucocorticoids

Glucocorticoids (GCs) are widely used anti-inflammatory drugs used to treat several inflammatory disorders, including pulmonary diseases such as persistent asthma, severe or exacerbated COPD, and chronic eosinophilic pneumonia.[139] GCs mediate their effects by binding to the GC receptor, which subsequently translocates into the nucleus. GC receptor can either act as a homodimeric transcription factor mediating the induction of several anti-inflammatory genes[140,141] or can modulate the activity of several other transcription factors, e.g., nuclear factor-κB or activator protein-1.[142,143] Synthesis of GCs occurs in the adrenal gland and serum concentrations are controlled by the adrenocorticotropic hormone. In recent years, nonadrenal tissues have also been identified to produce GCs. This is achieved either by *de novo* synthesis from cholesterol or by reactivation of inactive GC in a 11β-hydroxysteroid dehydrogenase (11β-HSD1)-dependent reaction.[144] Enzymes for the *de novo* synthesis of GC are expressed during embryonic lung organogenesis. In this context, GCs are important for proper lung development. Indeed, preterm mothers are given high doses of GCs antenatally to prevent respiratory distress syndrome of the neonate.[145] In line with this, GC receptor deficient mice display severely impaired lung function and die at birth.[146] 11β-HSD1 is also expressed in the fetal lung, yet seems to be less important for lung development as 11β-HSD1-deficient mice display a much less severe phenotype. Most likely, the loss of 11β-HSD1 is compensated by adrenal hyperplasia and increased adrenal GC secretion.[147] However, expression and GC synthesis by 11β-HSD1 has recently been reported to take place in adult murine lungs[148,149] and isolated murine airway epithelial cells.[150] Interestingly, pulmonary GC synthesis was elevated under inflammatory conditions, indicating a negative feedback mechanism.[149] Moreover, it was demonstrated that epithelial-derived GC, together with PGE_2, are able to induce tolerogenic dendritic cells.[119,150]

SWITCHING EPITHELIAL REGULATION UPON MICROBIAL CHALLENGE: INFLAMMATION IN THE PULMONARY SYSTEM

Given the above-mentioned mediators and modulatory mechanisms, we believe that the default setting in the lung is hyporesponsiveness or tolerance. However, the respiratory immune system has to actively switch to reactivity to allow clearance of pathogens that have established infections after overcoming the initial barriers. This is of special importance as only a few micrometers separate the interior of the body from the environment at the site of gas exchange. Indeed, several studies have demonstrated a proinflammatory role of epithelial cells during inflammation. Therefore, mechanisms must exist to overcome the tolerogenic state of homeostasis and allow epithelial cells to switch from tolerance into a reactive phenotype (**Figure 2**).

One possible mechanism would be a threshold regulation in which the absolute amount of antigen/PAMP accumulating in the lung is the decisive trigger. Physiologically, the upper respiratory tract filters out larger particles, which are subsequently swallowed and discarded into the gastrointestinal tract. However, smaller particles having an aerodynamic diameter below 2.5–3 μm are still able to reach the conducting airways or the lower respiratory tract.[37,151] These particles might precipitate in the conducting airways, become trapped in the mucus, and are cleared by the MCC. Disturbances of this transport results in chronic inflammatory processes such as CF, COPD, or ciliary dyskinesia. Similar observations have also been made in several mouse models affecting this process, e.g., Muc5 deficiency,[36] Scnn1β transgenic mice.[152] However, smaller particles can also reach the alveolar space. Here, these particles most likely get cleared by phagocytic alveolar macrophages. Yet, because of the steady-state inhibition of PRR signaling, these cells will not mount a strong or systemic immune reaction. However, if the amount of antigens inhaled or accumulated exceeds a potential threshold, this might lead to an activation of macrophages and the secretion of proinflammatory cytokines. Induction of inflammatory cytokines can downregulate inhibitory substances, as has been shown for surfactant,[49] resulting in a positive feedforward loop that becomes triggered once a certain threshold is overcome.

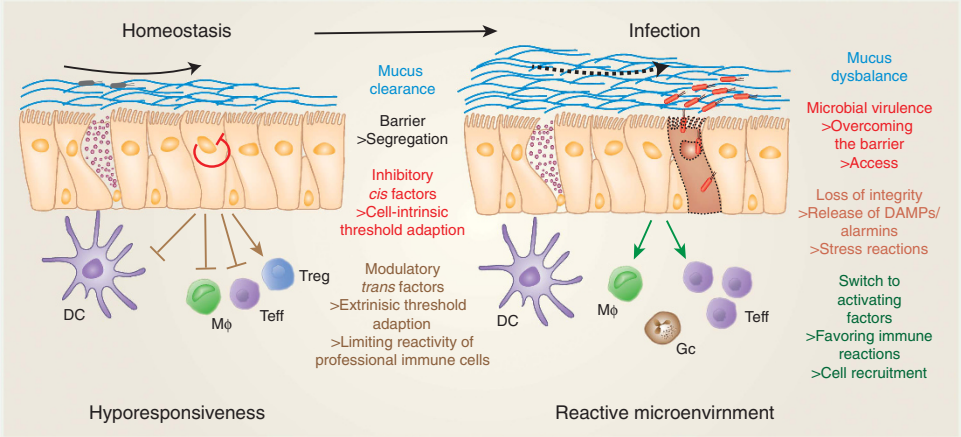

Figure 2 Epithelial cells regulate local immune reactions. Under homeostatic conditions airway epithelial cells promote a tolerogenic microenvironment. This is achieved by regulation of epithelial cells' sensor function in *cis* as well as the production of modulatory factors acting in *trans* on professional immune cells. Upon infectious encounter regulatory actions are reduced and proinflammatory activities induced generating a reactive microenvironment. Loss of epithelial cell integrity is a decisive checkpoint in this switching process.

Indeed, a specific role of alveolar macrophages in mounting pulmonary inflammatory processes has previously been proposed.[1,153] Their phagocytic capacity may be of special importance, whereby antigen uptake by alveolar macrophage would inhibit the transmission of these antigens to pulmonary dendritic cells.[154] By preventing the activation of dendritic cells, alveolar macrophages would be able to inhibit the activation of the adaptive immune system.[1]

Another mechanism could be based on the type of antigen or PAMP inhaled. It has been shown that airway epithelial cells are able to sense special PAMPs. Whereas airway epithelial cells are generally hyporesponsive towards prototypical PAMPs, including LPS or LTA, they do secrete proinflammatory cytokines upon poly-dI:dC challenge with the secretion of proinflammatory cytokines. This copies the clinical situation of bronchitis, which is usually caused by viruses but not by bacteria, resulting in an increased presence of viral PAMPs. Thus, it is possible that hyporesponsiveness is restricted to certain types of antigens/PAMPs. In this context, the concept of the so-called viability-associated (vita) PAMPs,[155] microbial molecules that only exist in viable microbes (e.g., bacterial mRNA), is an interesting alternative. Sensing mainly vita-PAMPs would mean that the inhalation of dead bacteria (characterized by a rapid loss of bacterial mRNA) does not elicit inflammation, whereas the presence of viable bacteria would result in activation. Here, specific PAMPs would act to signal infectious danger in a manner similar to vaccine adjuvants.

Another feature of many pulmonary pathogens is that they are able to induce cell death in airway epithelial cells. Therefore, interfering with epithelial cell integrity would, of course, result in lowering the above-mentioned immunomodulatory substances and the physical barrier function. In that case, inflammation would result from microbe-derived virulence factors destroying barrier cells and epithelium integrity, resulting in the release of inhibition. This would allow virulent microbes to reach the subepithelial compartment where they would encounter professional immune cells no longer suppressed by epithelial cell-derived substances. In addition, coordinated MCC needs an intact epithelial lining and disruption by epithelial cell loss might lead to the accumulation of antigen. Moreover, necrotic cell death leads to the release of several alarmins, including IL-33 and IL-1a,[106,107] both of which have been demonstrated to mount inflammatory responses.

Another cellular response able to induce proinflammatory cytokine secretion in epithelial cells is the unfolded protein response (UPR). UPR is activated upon endoplasmic reticulum stress, which occurs because of the accumulation of unfolded proteins in the endoplasmic lumen.[156] As chronic activation of UPR can also induce apoptosis, UPR combines several of the above-mentioned mechanism to induce inflammatory processes in the lung. Several agents are known to induce endoplasmic reticulum stress, including cigarette smoke,[157] bacteria,[158] and viruses.[159] Moreover, several chronic inflammatory pulmonary diseases have been linked to ER stress as well, including asthma, pulmonary fibrosis, and cystic fibrosis.[160–162] Nevertheless, the exact molecular role of UPR signaling under these conditions is still a matter of ongoing research.

CONCLUSIONS

Taken together, we have discussed evidence that airway epithelial cells are able to suppress inflammatory processes using various mechanisms. These include inhibition of endogenous PRR signaling as well as inhibition of professional leukocytes by cell–cell contacts or secretion of inhibitory substances (cytokines, eicosanoids, and GC). Hyporesponsiveness can be overcome upon infection with pathogens. Epithelial cell integrity will be an important checkpoint in this process, with downregulation of modulatory activities and upregulation of proinflammatory factors once epithelial cells are threatened by microbial infection. Therefore, airway

epithelial cells are a major player/modulator of airway and lung homeostasis and inflammation. Disturbance of these mechanisms may contribute to various chronic inflammatory diseases.

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DISCLOSURE

The authors declare no conflict of interest.

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