

REVIEW

Epithelial function and dysfunction in asthma

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Summary

Asthma was previously defined as an allergic Th2-mediated inflammatory immune disorder. Recently, this paradigm has been challenged because not all pathological changes observed in the asthmatic airways are adequately explained simply as a result of Th2-mediated processes. Contemporary thought holds that asthma is a complex immune disorder involving innate as well as adaptive immune responses, with the clinical heterogeneity of asthma perhaps a result of the different relative contribution of these two systems to the disease. Epidemiological studies show that exposure to certain environmental substances is strongly associated with the risk of developing asthma. The airway epithelium is first barrier to interact with, and respond to, environmental agents (pollution, viral infection, allergens), suggesting that it is a key player in the pathology of asthma. Epithelial cells play a key role in the regulation of tissue homeostasis by the modulation of numerous molecules, from antioxidants and lipid mediators to growth factors, cytokines, and chemokines. Additionally, the epithelium is also able to suppress mechanisms involved in, for example, inflammation in order to maintain homeostasis. An intrinsic alteration or defect in these regulation mechanisms compromises the epithelial barrier, and therefore, the barrier may be more prone to environmental substances and thus more likely to exhibit an asthmatic phenotype. In support of this, polymorphisms in a number of genes that are expressed in the bronchial epithelium have been linked to asthma susceptibility, while environmental factors may affect epigenetic mechanisms that can alter epithelial function and response to environmental insults. A detailed understanding of the regulatory role of the airway epithelium is required to develop new therapeutic strategies for asthma that not only address the symptoms but also the underlining pathogenic mechanism(s) and prevent airway remodelling.

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The burden of asthma

Henry Hyde Salter's seminal description of asthma as 'paroxysmal dyspnoea of a peculiar character, generally periodic, with intervals of healthy respiration between the attacks' still holds true today [1], with asthma being classified as a disease of the conducting airways, characterised by recurrent, reversible airflow obstruction, airways inflammation, bronchial hyperresponsiveness, and a progressive decline in lung function [2]. Unfortunately, the same cannot be said of his claim that asthma is 'not a very common disease' [1]. Recent estimates suggest that asthma affects at least 4.3% of the world-wide adult population [3]. The UK National Health Service spends approximately £1 billion per annum treating asthma [4], with £850 million alone

spent on drugs to control the disease [5]. However, there is still a major shortfall in the understanding and management of asthma, with controversy over the aetiology, pathophysiological processes, and treatment for asthma [6].

Asthma classification

The classification of asthma, ranging from intermittent through mild, moderate, and severe persistent forms, is based upon frequency and severity of exacerbations, and forced expiratory volume in 1 s (FEV₁) [7, 8]. However, asthma severity has traditionally been determined while the patient is not using medication, and new measures of asthma severity have been proposed, including the intensity of treatment required to bring

asthma under control [9]. Alternative classification methods, such as clinical and inflammatory phenotypes, endotype (pathophysiological mechanism), or trigger type, have been proposed with the aim of providing more targeted treatments into relevant subgroups of the disease [10–12]. Recent research has suggested that patients can be separated into clusters based on a small number of factors including baseline and maximum FEV₁ and age of onset [13]. It has also been suggested that clustering patients on the basis of age of onset and the extent of eosinophilic airway inflammation may enable more appropriate corticosteroid treatment, because non-eosinophilic asthma is more likely to be refractive to corticosteroids [14–16].

In addition to classification by phenotype, asthma can be subdivided into atopic and non-atopic asthma, with atopic asthma marked by increased Th2-type cytokines (e.g. IL-4, IL-5, IL-13). Although the majority of asthma cases are associated with atopy, a significant proportion are not [17]. Allergens which may trigger asthma include components of house dust mite faeces [18, 19], fungal spores [20–22], and pollen [23, 24]. Non-allergenic triggers include respiratory viral infections [25–27], air pollution including environmental cigarette smoke [28, 29], occupational exposures to a wide range of chemical and biological sensitizing agents and irritants [30], exercise [31], aspirin [32], and lifestyle factors such as stress and obesity [33–36].

The genetic basis of asthma

Despite much research, the causes of asthma are still incompletely understood. Asthma gene association studies are numerous, and genes positively associated with asthma fall into a number of distinct groups. Studies have implicated genes coding for inflammatory cytokines and their receptors, including those involved in Th2-type responses such as the *IL-4* gene cluster at chromosome 5q31 [37], *TSLP* [38, 39] and *IL-33* [40, 41], and the IL1 receptor gene cluster on chromosome 2q [42]. Other studies have identified genes involved in remodelling; for example, an association has been found between asthma (particularly bronchial hyperresponsiveness) and *ADAM33*, one of a subfamily of transmembrane metalloproteinases [43], which may contribute to structural changes by playing a role in angiogenesis [44]. Other genes appear able to play a role in epithelial function: *PCDH1* [45] and *CDHR3* [46], which may be involved in epithelial cell–cell adhesion, and, interestingly, the epidermal junction protein filaggrin *FLG1*, which has been strongly linked to development of atopic dermatitis [47], have also been linked to development of asthma [48, 49]. In addition, *ORMDL3* [50], which may control release of calcium from the endoplasmic reticulum in epithelial cells and T cells

[51, 52], and *GSDML* [53] are two genes at a locus on chromosome 17q21 that have also been linked to asthma. However, although there is a clear genetic link in asthma, twin studies have suggested that genetics alone cannot account for all or even most cases of asthma [54, 55], indicating that asthma is not determined purely by genetic factors, but rather by gene–environment interactions [56, 57]. The involvement of genes coding for molecules with such varying functions lends weight to the assertion that asthma is a multi-factorial disease with a range of aetiologies.

As many asthma-susceptibility genes are expressed in the airway epithelium, which is the first point of contact for any potential inhaled triggers, recent attention has focused on the airway epithelium as being crucial in the development and maintenance of an asthmatic phenotype [58, 59]. The remainder of this review will consider the function of the airway epithelium, and how aberrant epithelial function may act as a major contributor towards the development of asthma.

The airway epithelium

The airway epithelium provides a vital protective layer between the internal milieu of the lung and the external environment [60]. The bronchial epithelium has a pseudostratified structure, with the majority of cells being ciliated columnar epithelial cells, which fulfil a vital role in the clearance of inhaled pathogens and particles by the mucociliary escalator [61–63]. In addition, the columnar layer is populated by mucus-secreting goblet cells [64], neuroendocrine cells, and intermediate cells. Clara cells are also present, although more commonly found in the bronchioles, where they secrete pulmonary surfactant [65]. In humans, basal cells, which underlie the columnar epithelium, act as a pool of progenitor cells for the epithelium [66, 67]. Early thinking posited that the bronchial epithelium was merely an inert physical barrier against ingress of inhaled pathogens and toxins. However, it has since become apparent that the epithelium also provides a chemical and immunologic barrier between the airway submucosa and the external environment. Crucially, there is clear evidence that these functions may be perturbed in the bronchial epithelium of asthmatic subjects.

The epithelium as a physical barrier

Epithelial cells have a tendency to form sheets due to their propensity for forming adhesive contacts with adjacent epithelial cells, giving rise to a characteristic ‘cobblestone’ pattern [68, 69]. The set of epithelial cell–cell junctions closest to the airway lumen is the apical

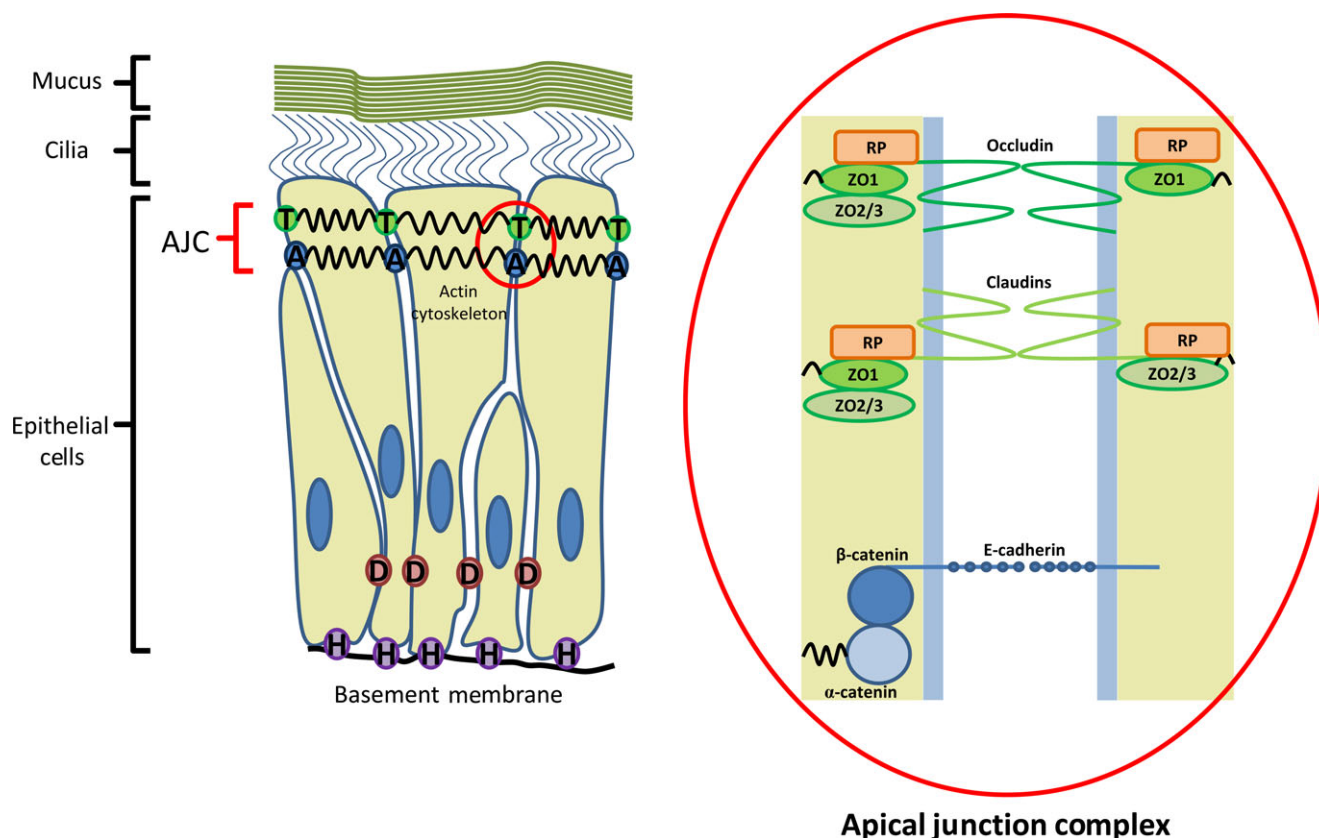


Fig. 1. Epithelial cell-cell junctions. Left: The function of the epithelium as a selectively permeable barrier against the ingress of pathogens and toxicants requires the presence of a variety of adhesion molecules, from the apical junction complex (AJC), which comprises the adherens junction (A) and tight junction (T), to the lateral desmosomes (D) and basolateral hemidesmosomes (H), which anchor the cells to the basement membrane. Right: The AJC is comprised of a variety of transmembrane junctional proteins and intracellular adapter molecules, linking the junctional plaques to the actin cytoskeleton, which may be modified by regulatory proteins (RP).

junction complex (Fig. 1), comprising the tight junction (TJ) and the adherens junction (AJ) [70]. The TJ is central to the function of the epithelium as a selectively permeable barrier: the 'gate' function, acting as a selectively but variably permeable barrier to the passage of ions and solutes *via* the paracellular pathway, and the 'fence' function, acting as a site of demarcation between the apical and basolateral domains of the cells membrane to define epithelial polarity [71]. TJs are composed of transmembrane proteins such as occludin [72] and claudins [73, 74], which are linked to the actin cytoskeleton by ZO-1, ZO-2, and, ZO-3 [75–77]. TJ permeability can be regulated by cytokines (e.g. TNF- α , IFN- γ , and interleukins) and growth factors *via* actions on actomyosin contractility and tight junction proteins [78]; different members of the claudin family confer distinctive ionic permeabilities on the TJ in which they are found, particularly determined by the first extracellular loop of the protein forming ion- and charge-selective pores [74, 79, 80]. For example, the presence of claudin-2 in the TJs has been seen to increase ionic permeability [81, 82], while claudin-4 increased barrier

tightness [83]. AJs, comprised of E-cadherin linked to the actin cytoskeleton *via* the α - and β -catenin adapter complex, are important in the development of the epithelial layer and TJ formation [84, 85]. Additional junctions in the epithelium include gap junctions which provide a link between the cytoplasm of adjacent cells, desmosomes, located at the base of the lateral surface of epithelial cells, hemidesmosomes, which anchor epithelial cells to the basement membrane [86], and integrins, heterodimeric receptors connected to the actin cytoskeleton which recognize and bind collagen and other components of the extracellular matrix [87].

Recent research has demonstrated that the barrier function of the airway epithelium is defective in asthma. In biopsies and differentiated bronchial epithelial cells obtained from severe asthmatic subjects, a post-transcriptional loss of junctional ZO-1 and occludin and increased barrier permeability were observed [88]. Furthermore, differentiated bronchial epithelial cells from severe asthmatic donors displayed increased epithelial macromolecular permeability and reduced E-cadherin, along with redistribution of the adapter

protein p120 catenin, which was ameliorated by a src-family kinase inhibitor [89].

The epithelium as a chemical barrier

The epithelium fulfils an important function in protecting the airway against ingress of noxious agents and pathogens, aided by airway mucus. Mucus is a highly proteinaceous mixture, rich in mucins [90] – extensively glycosylated proteins, with varying glycosylation states, cellular localization, and complex formation, suggestive of differing roles [91]. Secreted mucins (MUC2, MUC5AC, MUC5B, MUC6) form a gel layer above the periciliary fluid, while cell-surface mucins remain anchored to the cell membrane [92]. Crosslinking of the mucin glycoproteins forms a mesh-like barrier to prevent dehydration of the epithelial barrier, facilitate pathogen sequestration, and trap inhaled particulate matter by virtue of steric interactions based on the pore size of the mucin mesh, and electrostatic and hydrophobic interactions [93–95]. It has been suggested that the mucus provides effective protection against inhaled particles of a range of sizes, morphologies, and surface chemistries because the time required for penetration of particles through the mucus layer is much greater than the time needed for clearance of mucus-trapped particles by the mucociliary escalator [62].

The principal airway-secreted mucins are MUC5B from submucosal glands and MUC5AC from goblet cells, with MUC5B being the principal mucin under normal physiological conditions, and MUC5AC appearing to be the predominant up-regulated mucin in asthma, forming the basis of asthmatic mucus hypersecretion [96–99]. Control of MUC5AC expression is complex, but mouse and human cell culture models suggest that it is under the control of IL-13/IL-4 and EGFR pathways [100]. Mouse models implicate SAM pointed domain-containing ETS transcription factor (SPDEF) which can be up-regulated by IL-13 in a STAT6-dependent manner and which interacts with thyroid transcription factor-1 (TTF-1) to induce goblet cell hyperplasia and increased expression of MUC5AC and genes associated with protein glycosylation [101, 102]. Alternative inducers of mucus production include IL-1 β and TNF *via* p38 MAPK and ERK [103], and IL-17A by NF- κ B [104]. Pollutants such as cigarette smoke can also induce mucus production *via* the EGFR pathway [105, 106].

Airway lining fluid is rich in molecules which are critical in the defence against pathogens and pollutants. Airway antimicrobials include the cationic peptide β -defensins and LL37, and also lysozyme and lactoferrin, which target bacterial peptidoglycan and chelate iron, respectively [107]. Antioxidants are important in defending the airway epithelium against oxidative stress and may take the form of enzymes such as superoxide

dismutase (SOD), which converts superoxide to hydrogen peroxide, and catalase, which catabolizes hydrogen peroxide, or smaller molecules such as glutathione and urate. Other enzymes, such as glutathione peroxidase and haem oxygenase-1, do not have direct antioxidant effects but maintain the availability of antioxidants in the reduced state [108, 109].

The epithelium as an immunologic barrier

Although most inhaled substances are cleared without an inflammatory response, the airway epithelium fulfils a crucial role in the instigation of defence mechanisms against infection. Pattern recognition receptors (PRRs) recognize pathogen-associated molecular patterns (PAMPs), with Toll-like receptors (TLRs) being the most studied of the PRRs. TLRs each recognize specific ligands, for example TLR2 recognizes peptidoglycan of gram-positive bacteria [110, 111], TLR3 recognizes double-stranded viral RNA (dsRNA) [112], TLR4 recognizes amongst others lipopolysaccharide from gram-negative bacteria [113], and TLR7 is activated by single-stranded RNA [114]. Following activation, all Toll-like receptors except TLR3 signal *via* a MyD88/IRAK-dependent cascade resulting in activation of MAP kinases and nuclear translocation of NF- κ B [115]. TLR activation leads to the release of multiple pro-inflammatory cytokines and chemokines, initiating the inflammatory response. Other classes of epithelial cell receptors which may initiate the innate immune response include RIG-like helicases which detect dsRNA [116], protease-activated receptors (PARs) which are activated by proteolytic cleavage to allow activation by a tethered ligand [117], C-type lectins which bind carbohydrates on a range of pathogens and allergens [118], and NOD-like receptors which mediate the release of antimicrobial peptides [119].

Damage to the epithelium results in the release of endogenous damage-associated molecular patterns (DAMPs), termed 'alarmins', which can induce a Th2-type inflammatory response, [120–122]. For example, upon exposure to the protease-rich fungus *Alternaria alternata*, airway epithelial cells of naïve mice release ATP as an alarmin, which results in IL-33 release and development of a Th2 phenotype *via* the P2Y₂ receptor, although IL-33 itself may also be considered to be an alarmin [123, 124]. Furthermore, ATP has been seen to be raised in the airways of asthmatics, where it is able contribute to a Th2 phenotype by activating dendritic cells [125].

Structural changes in the epithelium

A characteristic feature of asthma is goblet cell hyperplasia and metaplasia, along with mucus hypersecretion, which has been observed in patients who have died of

an asthma attack, with the great majority of asthma deaths showing at least partial airway occlusion by mucus [126, 127]. Asthmatic airways show increased populations of mucus-secreting goblet cells not only in the central airways but also in the peripheral airways [128], this being ascribed to the transdifferentiation of ciliated epithelial cells and Clara cells, as opposed to goblet cell proliferation, under the control of a complex network of transcription factors [59]. Airway remodeling was originally thought to be a consequence of the inflammatory processes seen in asthma. However, it is now clear that this is not the case, because the *lamina reticularis* underlying the epithelium appears thickened in children prior to, and around, the time of diagnosis of asthma [129–131], and this is not necessarily the result of inflammatory processes [132], with structural changes probably following some early triggering factor, such as viral infection, in genetically susceptible individuals [133]. Moreover, it has recently been shown that epithelial compression leads to increased goblet cell numbers and subepithelial collagen deposition in the absence of increased inflammation [134]. It has been suggested that epithelial damage is responsible for the thickening of the *lamina reticularis* in asthma and that some of the genetic factors which predispose to asthma do so *via* a tendency towards increased epithelial damage. This is supported by *in vitro* studies which show increased susceptibility to oxidative stress [88, 135], fungal allergens [136], and respiratory viruses [137, 138].

It has been postulated that structural defects in the airways are fundamental to the pathogenesis of asthma, with an initial injury in susceptible epithelium inducing a cycle of damage and aberrant repair, resulting in an abnormal, 'immature' epithelium and increased allergen penetration and sensitization [139]. Asthmatic airways show increased levels of the cell cycle inhibitor p21^{waf}, potentially indicating a reduced ability of the asthmatic airways to repair after damage, which in normal airways is a multistep process involving migration of epithelial cells to the site of damage, followed by their differentiation into ciliated and secretory cells [140]. This abnormal repair is marked by increased deposition of collagen and matrix proteins, producing the aforementioned thickening of the *lamina reticularis* [141, 142]. Therefore, an event originating in the epithelium may have downstream consequences in other layers of the airway, and for the airways as a whole (Fig. 2).

Stress responses in airway epithelial cells

Oxidative stress is considered one of the main risk factors involved in asthma pathogenesis and driving asthma exacerbations. Many environmental substances have been shown to activate or interfere with pathways

regulated by oxidative stress, such as cigarette smoke [143], particulate air pollution [144, 145], and respiratory viruses [146–148]. Some asthmatic subjects may exhibit an intrinsic defect in antioxidant defence mechanisms suggesting an increased sensitivity to oxidative stress [135, 149]. Thus, noxious substances can induce oxidative stress and activate downstream events at levels which would be benign in non-asthmatic subjects. For example, polymorphisms in glutathione S-transferase (GST) genes are associated with increased asthma susceptibility [150]. Defective GST metabolism is thought to be associated with increased inflammatory responses to environmental substances, because *GSTM1* knockdown in bronchial epithelial cells caused an enhanced IL-8 and IL-1 β release after exposure to diesel particles [151]. Reactive oxygen species (ROS) can activate antioxidant defence mechanisms *via* the transcription factor Nrf2 [152] or influence chromatin accessibility by reducing histone deacetylase (HDAC) activity [153, 154]. Additionally, cellular stress results in increased levels of misfolded and unfolded proteins in the endoplasmic reticulum (ER), activating the unfolded protein response (UPR) to restore cell function or, if the damage is more extensive, induce apoptosis [155]. The UPR can promote inflammation [156] and fibrosis [157], and it is thought to be important in chronic lung disease [158]. In this context, it is noteworthy that transfection of *ORMDL3* in lung epithelial cells caused activation of ATF6 α and induced SERCA2b, suggesting that the ER UPR might be one pathway through which *ORMDL3* is linked to asthma [159].

Lipid mediators in asthma

The eicosanoid pathway is thought to play an important role in the regulation of epithelial homeostasis. Epithelium-derived prostaglandin E₂ (PGE₂) has been shown to attenuate the pro-inflammatory potential of dendritic cells by reducing their TNF- α and enhancing IL-10 release [160]. Furthermore, PGE₂ can act on airway smooth muscle to counteract bronchoconstriction [161] and reverse myofibroblast differentiation by reducing α -SMA expression [162]. In the lung, these anti-inflammatory actions and bronchodilation caused by PGE₂ are thought to be mediated by E-prostanoid 2 (EP2) and/or EP4 receptors [163]. Eicosanoid synthesis can involve a transcellular mechanism with precursors generated by one cell type and then passed onto another cell type for further processing. For example, the synthesis of lipoxin A₄ (LXA₄), a counteracting eicosanoid which resolves inflammation, by the 5-/15-LO pathway involves generation of 15S-hydroxyperoxy-eicosatetraenoic acid (15S-H(p)ETE) by 15-LO in epithelial cells. After export, this intermediate is further processed by infiltrating immune cells into LXA₄ mediated by 5-LO [164].

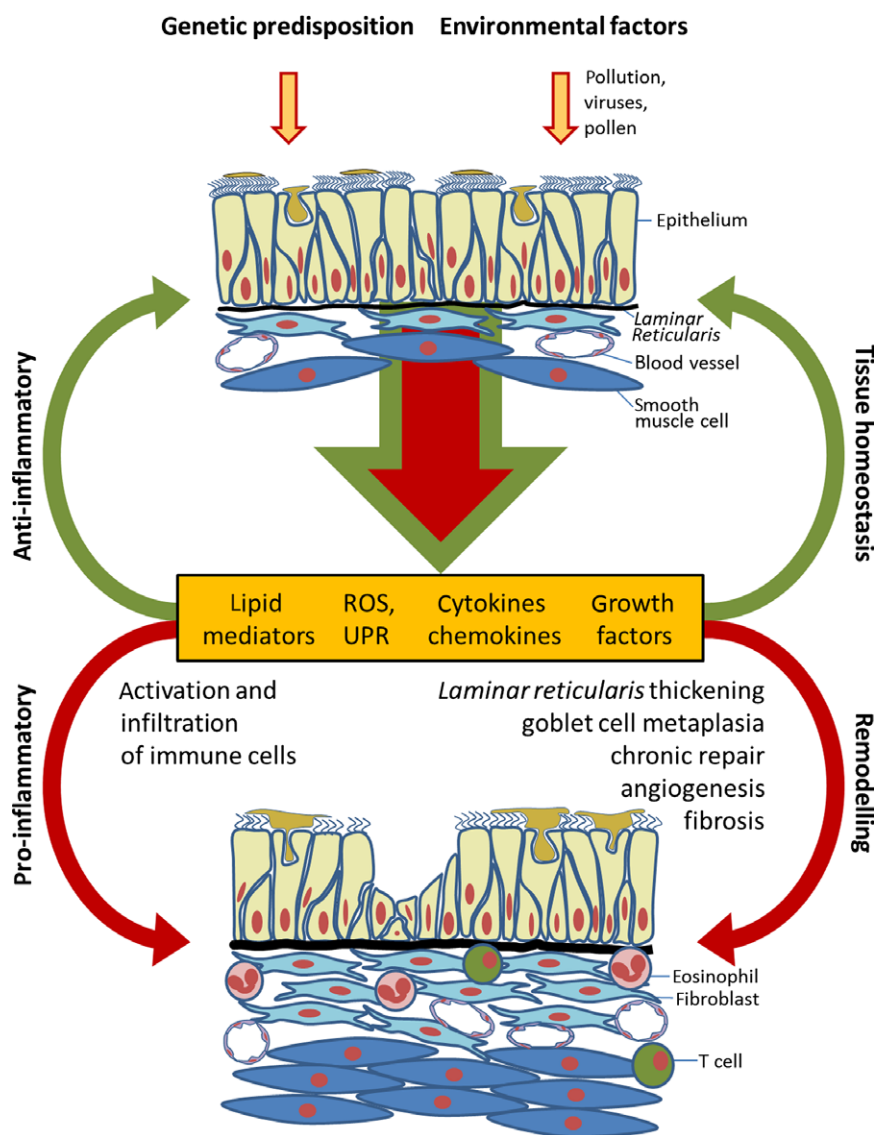


Fig. 2. The regulatory role of the airway epithelium. The functions of the bronchial epithelium are influenced by gene–environment interactions. In response to environmental factors, the bronchial epithelium can respond with the release or suppression of various mediators such as lipid mediators, stress response including reactive oxygen species (ROS), unfolded protein response (UPR), cytokines, chemokines, and growth factors. Some of these mediators can activate anti-inflammatory responses and contribute to tissue homeostasis by inducing repair mechanisms. On the other hand, some mediators are pro-inflammatory by inducing the activation and infiltration of immune cells into the airway mucosa or induce airway remodelling and thus contribute to the asthma phenotype.

Resolvins are a further class of lipid mediators thought to be involved in the resolution of airway inflammation [165].

The balance between pro- and anti-inflammatory lipid mediators appears disrupted in asthmatic subjects, favouring chronic airway inflammation, airflow obstruction, or remodelling [166, 167]. In asthma, there are increased levels of leukotriene B₄ (LTB₄) and cysteinyl leukotrienes (CysLTs), potent pro-inflammatory lipid mediators which also mediate bronchoconstriction [168]. The key enzyme in leukotriene synthesis is 5-LO, which is mainly expressed by cells of myeloid origin.

Airway epithelial cells may be able to interfere indirectly with the leukotriene pathway especially in asthmatic subjects. For example, transglutaminase 2 is overexpressed in the asthmatic airway epithelium and is able to increase the activity of secreted phospholipase A₂ group X (sPLA₂-X) [169]. It has also been shown that secreted phospholipase enhances leukotriene production in eosinophils [170] linking airway epithelial cells to leukotriene metabolism. However, the involvement of the airway epithelium in eicosanoid metabolism or regulation of epithelial cell function by eicosanoids is not fully understood.

Cytokines and chemokines in airway inflammation

Cytokines and chemokines released by airway epithelial cells activate and modulate the function of immune cells and structural cells and also act as chemoattractants. Furthermore, epithelial cells express many receptors for cytokines and chemokines, activation of which can influence epithelial functions. Cytokines linked to Th2-mediated immune responses, such as IL-4, IL-13, IL-25, IL-33, and TSLP, are crucial in the pathogenesis of atopic asthma. The release and function of these cytokines are reviewed elsewhere [171–174]. An intrinsically altered baseline or allergen/toxicant-stimulated release of cytokines and chemokines by airway epithelial cells derived from asthmatic individuals has been shown in many studies [137, 175–178]. Genetic polymorphisms may be one reason for an altered cytokine release in asthmatic epithelial cells [151]. It is also hypothesized that in asthma the expression of cytokines and chemokines is altered by epigenetic mechanisms [179]. These influence the activity of genes without changes in the DNA base sequence, including DNA methylation and histone acetylation/deacetylation, which alter the structural conformation and accessibility of DNA. For example, IL-17A mediates reduced activity of HDAC2, which is linked to a reduction in the glucocorticoid sensitivity of TNF- α -induced IL-8 release in bronchial epithelial cells [180]. However, the role of HDAC2 activity in glucocorticoid insensitive asthma is still under discussion [181, 182].

One of the most studied environmental impacts on the airway epithelium is viral infection, showing the important function of the airway epithelium as a link between innate and adaptive immune responses. It has been hypothesized that the innate antiviral response of the airway epithelium in asthmatic subjects is diminished, for example *in vitro* studies showed reduced release of interferon (IFN) after viral infection [138, 183, 184]. However, this is controversial because other studies showed no difference in virus-induced IFN-release between non-asthmatic and asthmatic subjects [185, 186]. This difference could be explained by the use of mild asthmatic subjects in the latter studies, whereas in the earlier studies, bronchial epithelial cells were derived from moderate-to-severe asthmatic subjects. Epithelial release of IFN activates the expression of IFN-stimulated genes (ISGs) priming antiviral defence mechanisms in neighbouring cells [187], including airway epithelial cells, fibroblasts and immune cells. A deficiency in IFN signalling compromises the antiviral defence mechanisms, which may contribute to the increased severity and duration of lower respiratory tract infections observed in asthmatic individuals [188]. Epidemiological studies detected a link between viral infections in early life and asthma

susceptibility; however, the mechanisms are still under investigation [189]. It is suggested that viral infections in early life promote structural changes in the airways which make the airway epithelium more prone to other environmental impacts such as allergens, favouring chronic airway inflammation and remodelling [190].

Growth factors and other proteins involved in airway remodelling

Airway remodelling, a central part of asthma pathogenesis, is thought to be mediated by growth factors and other proteins which are able to induce structural changes in the airways such as fibrosis, goblet cell hyperplasia, and angiogenesis. Transforming growth factor- β (TGF- β) has a central function in airway remodelling [191, 192]. The release of TGF- β by airway epithelial cells can be induced by environmental stimuli as well as intrinsic factors, and it has been shown that airway epithelial cells of asthmatic subjects release higher levels of TGF- β than non-asthmatics [193]. Additionally, airway epithelial cell function is modulated by TGF- β [194], for example the induction of epithelial-mesenchymal transition [195]. TGF- β inhibits the antiviral IFN- β response in bronchial epithelial cells, promoting rhinovirus replication [196]. Intracellular signalling pathways of TGF- β involve Smad2/3, and Smad3 polymorphisms are associated with an increased risk of asthma [41]. It has also been observed that rhinovirus-infected bronchial epithelial cells release basic fibroblast growth factor (bFGF), which activates fibroblasts [197]. Furthermore, rhinovirus infection causes the release of vascular endothelial growth factor (VEGF) by airway epithelial cells [198]. In addition to VEGF [199], epidermal growth factor (EGF) is an important factor in airway remodelling [194, 200]. EGF is normally sequestered on the basolateral side of the epithelium, away from apical EGFR, but perturbation of the epithelial barrier, such as by proteolytic allergens, allows access of EGF to its receptor, inducing epithelial activation [59].

One of the most prominent features of asthma is the changed properties of the lung extracellular matrix (ECM) associated with tissue stiffness and elasticity. The composition of the ECM is altered in asthma, mainly by an imbalance in its deposition and degradation [194, 201]. One important class of ECM-degrading enzymes are matrix metalloproteinases (MMPs) and their inhibitors, and tissue inhibitors of metalloproteinases (TIMPs) [202]. Environmental insults can interfere with the MMP/TIMP balance – it has been shown that rhinovirus infection induces MMP-9 expression in airway epithelial cells [203]. However, the role of the epithelium in such regulatory mechanisms is unknown. It has been suggested that periostin, which is expressed in

bronchial epithelial cells in response to IL-13, activates the TGF- β pathway in an MMP-dependent manner to cause fibrosis [204].

Perspective for new therapeutic strategies

Current asthma therapies suppress the symptoms and airway inflammation without targeting the underlying cause of the disease. Inhaled β_2 -adrenoceptor agonists induce relaxation of bronchial smooth muscle thereby increasing airway patency. They are used as prophylaxis or 'relievers', which may be short-acting (e.g. salbutamol) or long-acting (e.g. salmeterol) [205]. Corticosteroids, which may be inhaled or orally administered, act *via* the nuclear glucocorticoid receptor to reduce the airway inflammation [206]. Cysteinyl leukotriene pathway antagonists (e.g. montelukast) block the effects of cysteinyl leukotrienes, lipid mediators with an emerging role in the pathogenesis of asthma [168]. Finally, omalizumab is a humanized monoclonal antibody against IgE, levels of which are typically raised in atopic asthma. However, although omalizumab exhibits clear benefit in the treatment of a subset of patients with asthma, its use may be limited due to the cost of treatment [207,208]. As a result, there is an unmet clinical need for new therapeutic strategies, in particular targeting airway remodelling, especially for patients with severe forms of asthma. Due to the marked heterogeneity of asthma pathogenesis, there is a need for patient-tailored therapies and a requirement for specific biomarkers to identify susceptible individuals [209]. Additionally, experimental animal models commonly used to identify new therapeutic strategies have largely failed to translate into clinical advances. This failure is thought to be due to the poor recapitulation of the airway remodelling component of asthma, development over the course of life, and airway physiology of such animal models, which mainly focus on allergic airway inflammation [210,211]. Therefore, there is a need for experimental human-based models recapitulating the full complexity of the airway mucosa [212].

Currently, promising targets for asthma therapy are thought to modulate the epithelial response to environmental substances, preventing further downstream events such as airway remodelling and inflammation. Addressing antioxidant defence mechanisms might be of benefit for a subgroup of asthmatic patients, although dietary supplementation of antioxidants has previously failed to show beneficial effects in improving asthma control [213]. Targeting the Nrf2 signalling pathway may prove beneficial in reducing airway oxidative stress in asthma. *In vitro* studies suggest that resveratrol can modulate the response of airway epithelial cells to environmental substances by activating the Nrf2 pathway [214–216], but a better understanding of

the mechanisms involved is required before clinical trials can be performed. Another promising strategy is the targeting of eicosanoid metabolism to restore the balance between pro- and anti-inflammatory lipid mediators. In addition to currently available LT receptor antagonists such as montelukast, studies are investigating the inhibition of LT synthesis by blocking either 5-LO or 5-LO-activating protein (FLAP). However, blockade of 5-LO activity might also affect the synthesis of anti-inflammatory lipid mediators [217]. Modulation of the cyclooxygenase (COX) pathway of eicosanoid synthesis may prove beneficial for some asthmatic patients [218]. Other strategies include the development of synthetic EP4 receptor agonists promoting bronchodilation [219]. Anti-inflammatory lipid mediators as well as synthetic metabolically stable mimetics are also being investigated as possible asthma therapies [220,221], with an *in vitro* study having shown that lipoxin A4 can improve the physical barrier properties of bronchial epithelial cells [222].

Antibody treatments are also being tested as potential asthma therapeutics, with IL-4, IL-5, and IL-13 being promising targets for such treatments. However, clinical trials have produced mixed results, perhaps due to the great degree of heterogeneity of asthma. One study showed that anti-IL-13 monoclonal antibody therapy is most effective in a subgroup of patients with a high Th2 background and high periostin levels, suggesting periostin as a biomarker to identify responsive patients [223]. IL-4 receptor α polymorphisms are thought to predict a subgroup of patients with asthma which are more responsive to IL-4 receptor α antagonist therapy [224]. A recent study has shown the effectiveness of dupilumab, an antibody against the α -subunit of the IL-4 receptor, in the treatment of moderate-to-severe asthma patients with elevated eosinophil levels [225]. Targeting eosinophilia in asthma with anti-IL-5 (mepolizumab) has also been shown to reduce asthma exacerbations but without improving lung function significantly [226].

Restoration of the physical barrier of the epithelium may also prove fruitful. It has been shown *in vitro* that EGF can restore the physical barrier in asthmatic subjects [88]. Keratinocyte growth factor (KGF), which is able to increase alveolar barrier functions *in vitro* [227], has also been discussed as a treatment for acute lung injury and might also be beneficial for asthmatic patients with reduced physical barrier functions. Another promising strategy to reduce asthma exacerbations is to target viral infections. *In vitro*, exogenous IFN- β can restore the antiviral defence mechanisms of bronchial epithelial cells from patients with asthma [228], suggesting that exogenous IFN- β may reduce the severity of asthma exacerbations and might also be beneficial for early life rhinoviral infections and reducing

the risk of asthma development. Clinical trials using inhaled IFN- β during respiratory viral infections in asthma have been undertaken and will report soon.

Conclusion

To date, the underlining pathomechanisms of asthma are not fully understood, but it is evident that asthma is a heterogeneous immune disorder involving innate and adaptive immune responses as a result of complex gene–environment interactions. As the airway epithelium is the first line of contact to environmental

substances, and genome-wide association studies identified several genes associated with asthma expressed in the airway epithelium, it plays a key role in the pathomechanisms of asthma. A detailed understanding of the regulatory role of the airway epithelium in health and disease will help to identify subtypes of asthma and develop new patient-tailored therapeutic strategies.

Conflict of interest

DED is founder, consultant, and shareholder in Synairgen Ltd. ML and CB have no competing interests.

References

- 1 Salter HH. *On asthma: its pathology and treatment*. Philadelphia: Blanchard and Lea, 1860.
- 2 Lemanske RF, Busse WW. Asthma: clinical expression and molecular mechanisms. *J Allergy Clin Immunol* 2010; 125:S95–102.
- 3 To T, Stanojevic S, Moores G *et al*. Global asthma prevalence in adults: findings from the cross-sectional world health survey. *BMC Public Health* 2012; 12:204.
- 4 Asthma UK. Asthma facts and FAQs, 2013.
- 5 National Health Service UK. What is asthma? 2013.
- 6 Holgate S, Bisgaard H, Bjermer L *et al*. The Brussels Declaration: the need for change in asthma management. *Eur Respir J* 2008; 32: 1433–42.
- 7 British Thoracic Society Scottish Intercollegiate Guidelines Network. British guideline on the management of asthma. *Thorax* 2008; 63: iv1–121.
- 8 Yawn BP. Factors accounting for asthma variability: achieving optimal symptom control for individual patients. *Prim Care Respir J* 2008; 17:138–47.
- 9 Taylor DR, Bateman ED, Boulet LP *et al*. A new perspective on concepts of asthma severity and control. *Eur Respir J* 2008; 32:545–54.
- 10 Agache IO. From phenotypes to endotypes to asthma treatment. *Curr Opin Allergy Clin Immunol* 2013; 13:249–56.
- 11 Wenzel SE. Asthma: defining of the persistent adult phenotypes. *Lancet* 2006; 368:804–13.
- 12 Lotvall J, Akdis CA, Bacharier LB *et al*. Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. *J Allergy Clin Immunol* 2011; 127: 355–60.
- 13 Moore WC, Meyers DA, Wenzel SE *et al*. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2010; 181:315–23.
- 14 Haldar P, Pavord ID, Shaw DE *et al*. Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med* 2008; 178: 218–24.
- 15 Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavord ID. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. *Thorax* 2002; 57:875–9.
- 16 Pavord ID, Brightling CE, Woltmann G, Wardlaw AJ. Non-eosinophilic corticosteroid unresponsive asthma. *Lancet* 1999; 353:2213–4.
- 17 Holgate ST, Arshad HS, Roberts GC, Howarth PH, Thurner P, Davies DE. A new look at the pathogenesis of asthma. *Clin Sci* 2010; 118:439–50.
- 18 Sporik R, Holgate ST, Plattsmills TAE, Cogswell JJ. Exposure to house dust mite allergen (Der p 1) and the development of asthma in childhood – a prospective study. *N Engl J Med* 1990; 323: 502–7.
- 19 Hammad H, Chieppa M, Perros F, Willart MA, Germain RN, Lambrecht BN. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat Med* 2009; 15: 410–6.
- 20 Black PN, Udy AA, Brodie SM. Sensitivity to fungal allergens is a risk factor for life-threatening asthma. *Allergy* 2000; 55:501–4.
- 21 O'Driscoll BR, Hopkinson LC, Denning DW. Mold sensitization is common amongst patients with severe asthma requiring multiple hospital admissions. *BMC Pulm Med* 2005; 5:4.
- 22 Denning DW, O'Driscoll BR, Hogabam CM, Bowyer P, Niven RM. The link between fungi and severe asthma: a summary of the evidence. *Eur Respir J* 2006; 27:615–26.
- 23 Darrow LA, Hess J, Rogers CA, Tolbert PE, Klein M, Sarnat SE. Ambient pollen concentrations and emergency department visits for asthma and wheeze. *J Allergy Clin Immunol* 2012; 130:630–8.
- 24 Dales RE, Cakmak S, Judek S *et al*. Influence of outdoor aeroallergens on hospitalization for asthma in Canada. *J Allergy Clin Immunol* 2004; 113:303–6.
- 25 Miller EK, Edwards KM, Weinberg GA *et al*. A novel group of rhinoviruses is associated with asthma hospitalizations. *J Allergy Clin Immunol* 2009; 123:98–104.
- 26 Papadopoulos NG, Xepapadaki P, Mallia P *et al*. Mechanisms of virus-induced asthma exacerbations: state-of-the-art. A GA(2)LEN and InterAirways document. *Allergy* 2007; 62:457–70.
- 27 Johnston NW, Johnston SL, Duncan JM *et al*. The September epidemic of asthma exacerbations in children: a search for etiology. *J Allergy Clin Immunol* 2005; 115:132–8.
- 28 Althuis MD, Sexton M, Prybylski D. Cigarette smoking and asthma symptom severity among adult asthmatics. *J Asthma* 1999; 36:257–64.
- 29 Li N, Hao MQ, Phalen RF, Hinds WC, Nel AE. Particulate air pollutants and asthma – a paradigm for the role of oxidative stress in PM-induced

- adverse health effects. *Clin Immunol* 2003; **109**:250–65.
- 30 Dykewicz MS. Occupational asthma: current concepts in pathogenesis, diagnosis, and management. *J Allergy Clin Immunol* 2009; **123**:519–28.
 - 31 Anderson SD, Daviskas E. The mechanism of exercise-induced asthma is... *J Allergy Clin Immunol* 2000; **106**:453–9.
 - 32 Stevenson DD, Szczeklik A. Clinical and pathologic perspectives on aspirin sensitivity and asthma. *J Allergy Clin Immunol* 2006; **118**:773–86.
 - 33 Farah CS, Salome CM. Asthma and obesity: a known association but unknown mechanism. *Respirology* 2012; **17**:412–21.
 - 34 Sutherland ER, Goleva E, King TS *et al.* Cluster analysis of obesity and asthma phenotypes. *PLoS ONE* 2012; **7**:e36631.
 - 35 Fenger RV, Gonzalez-Quintela A, Vidal C *et al.* Exploring the obesity-asthma link: do all types of adiposity increase the risk of asthma? *Clin Exp Allergy* 2012; **42**:1237–45.
 - 36 Loerbroeks A, Gadinger MC, Bosch JA, Sturmer T, Amelang M. Work-related stress, inability to relax after work and risk of adult asthma: a population-based cohort study. *Allergy* 2010; **65**:1298–305.
 - 37 Ober C, Cox NJ, Abney M *et al.* Genome-wide search for asthma susceptibility loci in a founder population. *Hum Mol Genet* 1998; **7**:1393–8.
 - 38 Harada M, Hirota T, Jodo AI *et al.* Thymic Stromal lymphopoietin gene promoter polymorphisms are associated with susceptibility to bronchial asthma. *Am J Respir Cell Mol Biol* 2011; **44**:787–93.
 - 39 He JQ, Hallstrand TS, Knight D *et al.* A thymic stromal lymphopoietin gene variant is associated with asthma and airway hyperresponsiveness. *J Allergy Clin Immunol* 2009; **124**:222–9.
 - 40 Gudbjartsson DF, Bjornsdottir US, Halapi E *et al.* Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat Genet* 2009; **41**:342–7.
 - 41 Moffatt MF, Gut IG, Demenais F *et al.* A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med* 2010; **363**:1211–21.
 - 42 Reijmerink NE, Postma DS, Bruinenberg M *et al.* Association of IL1RL1, IL18R1, and IL18RAP gene cluster polymorphisms with asthma and atopy. *J Allergy Clin Immunol* 2008; **122**:651–4.
 - 43 Van Eerdewegh P, Little RD, Dupuis J *et al.* Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* 2002; **418**:426–30.
 - 44 Puxeddu I, Pang YY, Harvey A *et al.* The soluble form of a disintegrin and metalloprotease 33 promotes angiogenesis: implications for airway remodeling in asthma. *J Allergy Clin Immunol* 2008; **121**:1400–6.
 - 45 Koppelman GH, Meyers DA, Howard TD *et al.* Identification of PCDH1 as a novel susceptibility gene for bronchial hyperresponsiveness. *Am J Respir Crit Care Med* 2009; **180**:929–35.
 - 46 Bonnelykke K, Sleiman P, Nielsen K *et al.* A genome-wide association study identifies CDHR3 as a susceptibility locus for early childhood asthma with severe exacerbations. *Nat Genet* 2014; **46**:51–5.
 - 47 Palmer CNA, Irvine AD, Terron-Kwiatkowski A *et al.* Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006; **38**:441–6.
 - 48 Weidinger S, O'Sullivan M, Illig T *et al.* Filaggrin mutations, atopic eczema, hay fever, and asthma in children. *J Allergy Clin Immunol* 2008; **121**:1203–9.
 - 49 McLean WHI, Palmer CNA, Henderson J, Kabesch M, Weidinger S, Irvine AD. Filaggrin variants confer susceptibility to asthma. *J Allergy Clin Immunol* 2008; **121**:1294–5.
 - 50 Moffatt MF, Kabesch M, Liang LM *et al.* Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature* 2007; **448**:470–3.
 - 51 Cantero-Recasens G, Fandos C, Rubio-Moscardo F, Valverde MA, Vicente R. The asthma-associated ORMDL3 gene product regulates endoplasmic reticulum-mediated calcium signaling and cellular stress. *Hum Mol Genet* 2010; **19**:111–21.
 - 52 Carreras-Sureda A, Cantero-Recasens G, Rubio-Moscardo F *et al.* ORMDL3 modulates store-operated calcium entry and lymphocyte activation. *Hum Mol Genet* 2013; **22**:519–30.
 - 53 Wu H, Romieu I, Sienra-Monge JJ, Li H, del Rio-Navarro BE, London SJ. Genetic variation in ORM1-like 3 (ORMDL3) and gasdermin-like (GSDML) and childhood asthma. *Allergy* 2009; **64**:629–35.
 - 54 Nieminen MM, Kaprio J, Koskenvuo M. A population-based study of bronchial-asthma in adult twin pairs. *Chest* 1991; **100**:70–5.
 - 55 Skadhauge LR, Christensen K, Kyvik KO, Sigsgaard T. Genetic and environmental influence on asthma: a population based study of 11,688 Danish twin pairs. *Eur Respir J* 1999; **13**:8–14.
 - 56 von Mutius E. Gene-environment interactions in asthma. *J Allergy Clin Immunol* 2009; **123**:3–11.
 - 57 Ober C, Vercelli D. Gene-environment interactions in human disease: nuisance or opportunity? *Trends Genet* 2011; **27**:107–15.
 - 58 Holgate ST. The airway epithelium is central to the pathogenesis of asthma. *Allergol Int* 2008; **57**:1–10.
 - 59 Lambrecht BN, Hammad H. The airway epithelium in asthma. *Nat Med* 2012; **18**:684–92.
 - 60 Holgate ST. The sentinel role of the airway epithelium in asthma pathogenesis. *Immunol Rev* 2011; **242**:205–19.
 - 61 Knight DA, Holgate ST. The airway epithelium: structural and functional properties in health and disease. *Respirology* 2003; **8**:432–46.
 - 62 Kirch J, Guenther M, Doshi N *et al.* Mucociliary clearance of micro- and nanoparticles is independent of size, shape and charge-an ex vivo and in silico approach. *J Control Release* 2012; **159**:128–34.
 - 63 Antunes MB, Cohen NA. Mucociliary clearance - a critical upper airway host defense mechanism and methods of assessment. *Curr Opin Allergy Clin Immunol* 2007; **7**:5–10.
 - 64 Rogers DF. The airway goblet cell. *Int J Biochem Cell B* 2003; **35**:1–6.
 - 65 Reynolds SD, Malkinson AM. Clara cell: progenitor for the bronchiolar epithelium. *Int J Biochem Cell B* 2010; **42**:1–4.
 - 66 Rock JR, Onaitis MW, Rawlins EL *et al.* Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc Natl Acad Sci USA* 2009; **106**:12771–5.

- 67 Hong KU, Reynolds SD, Watkins S, Fuchs E, Stripp BR. Basal cells are a multipotent progenitor capable of renewing the bronchial epithelium. *Am J Pathol* 2004; 164:577–88.
- 68 Cozens AL, Yezzi MJ, Kunzelmann K *et al.* CFTR expression and chloride secretion in polarized immortal human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 1994; 10:38–47.
- 69 Boulblil L, Assemet E, Borot MC *et al.* Development of a repeated exposure protocol of human bronchial epithelium in vitro to study the long-term effects of atmospheric particles. *Toxicol In Vitro* 2013; 27:533–42.
- 70 Bruewer M, Luegering A, Kucharzik T *et al.* Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. *J Immunol* 2003; 171:6164–72.
- 71 Sawada N, Murata M, Kikuchi K *et al.* Tight junctions and human diseases. *Med Electron Microsc* 2003; 36:147–56.
- 72 Furuse M, Hirase T, Itoh M, Nagafuchi A, Yonemura S, Tsukita S. Occludin: a novel integral membrane protein localizing at tight junctions. *J Cell Biol* 1993; 123:1777–88.
- 73 Morita K, Furuse M, Fujimoto K, Tsukita S. Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands. *Proc Natl Acad Sci USA* 1999; 96:511–6.
- 74 Krause G, Winkler L, Mueller SL, Haseloff RF, Piontek J, Blasig IE. Structure and function of claudins. *Biochim Biophys Acta* 2008; 1778:631–45.
- 75 Fanning AS, Anderson JM. Zonula occludens-1 and-2 are cytosolic scaffolds that regulate the assembly of cellular junctions. *Ann N Y Acad Sci* 2009; 1165:113–20.
- 76 Fanning AS, Jameson BJ, Jesaitis LA, Anderson JM. The tight junction protein ZO-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton. *J Biol Chem* 1998; 273:29745–53.
- 77 Fanning AS, Van Itallie CM, Anderson JM. Zonula occludens-1 and-2 regulate apical cell structure and the zonula adherens cytoskeleton in polarized epithelia. *Mol Biol Cell* 2012; 23:577–90.
- 78 Steed E, Balda MS, Matter K. Dynamics and functions of tight junctions. *Trends Cell Biol* 2010; 20:142–9.
- 79 Van Itallie CM, Anderson JM. Claudins and epithelial paracellular transport. *Annu Rev Physiol* 2006; 68:403–29.
- 80 Van Itallie CM, Fanning AS, Anderson JM. Reversal of charge selectivity in cation or anion-selective epithelial lines by expression of different claudins. *Am J Physiol-Renal* 2003; 285: F1078–84.
- 81 Furuse M, Furuse K, Sasaki H, Tsukita S. Conversion of zonulae occludentes from tight to leaky strand type by introducing claudin-2 into Madin-Darby canine kidney I cells. *J Cell Biol* 2001; 153:263–72.
- 82 Amasheh S, Meiri N, Gitter AH *et al.* Claudin-2 expression induces cation-selective channels in tight junctions of epithelial cells. *J Cell Sci* 2002; 115:4969–76.
- 83 Van Itallie C, Rahner C, Anderson JM. Regulated expression of claudin-4 decreases paracellular conductance through a selective decrease in sodium permeability. *J Clin Invest* 2001; 107:1319–27.
- 84 Knust E, Bossinger O. Composition and formation of intercellular junctions in epithelial cells. *Science* 2002; 298:1955–9.
- 85 Baum B, Georgiou M. Dynamics of adherens junctions in epithelial establishment, maintenance, and remodeling. *J Cell Biol* 2011; 192:907–17.
- 86 Green KJ, Jones JC. Desmosomes and hemidesmosomes: structure and function of molecular components. *FASEB J* 1996; 10:871–81.
- 87 Barczyk M, Carracedo S, Gullberg D. Integrins. *Cell Tissue Res* 2010; 339:269–80.
- 88 Xiao C, Puddicombe SM, Field S *et al.* Defective epithelial barrier function in asthma. *J Allergy Clin Immunol* 2011; 128:549–56.
- 89 Hardyman MA, Wilkinson E, Martin E *et al.* TNF- α -mediated bronchial barrier disruption and regulation by src-family kinase activation. *J Allergy Clin Immunol* 2013; 132:665–75.e8.
- 90 Nicholas B, Skipp P, Mould R *et al.* Shotgun proteomic analysis of human-induced sputum. *Proteomics* 2006; 6:4390–401.
- 91 Williams OW, Sharafkhaneh A, Kim V, Dickey BF, Evans CM. Airway mucus – from production to secretion. *Am J Respir Cell Mol Biol* 2006; 34:527–36.
- 92 Cone RA. Barrier properties of mucus. *Adv Drug Deliver Rev* 2009; 61:75–85.
- 93 Lai SK, Wang YY, Hanes J. Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. *Adv Drug Deliver Rev* 2009; 61:158–71.
- 94 Jachak A, Lai SK, Hida K *et al.* Transport of metal oxide nanoparticles and single-walled carbon nanotubes in human mucus. *Nanotoxicology* 2012; 6:614–22.
- 95 Thornton DJ, Rousseau K, McGuckin MA. Structure and function of the polymeric mucins in airways mucus. *Annu Rev Physiol* 2008; 70:459–86.
- 96 Evans CM, Kim K, Tuvim MJ, Dickey BF. Mucus hypersecretion in asthma: causes and effects. *Curr Opin Pulm Med* 2009; 15:4–11.
- 97 Kirkham S, Sheehan JK, Knight D, Richardson PS, Thornton DJ. Heterogeneity of airways mucus: variations in the amounts and glycoforms of the major oligomeric mucins MUC5AC and MUC5B. *Biochem J* 2002; 361:537–46.
- 98 Voynow JA, Gendler SJ, Rose MC. Regulation of mucin genes in chronic inflammatory airway diseases. *Am J Respir Cell Mol Biol* 2006; 34:661–5.
- 99 Rose MC, Voynow JA. Respiratory tract mucin genes and mucin glycoproteins in health and disease. *Physiol Rev* 2006; 86:245–78.
- 100 Kuperman DA, Huang XZ, Koth LL *et al.* Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. *Nat Med* 2002; 8:885–9.
- 101 Park KS, Korfhagen TR, Bruno MD *et al.* SPDEF regulates goblet cell hyperplasia in the airway epithelium. *J Clin Invest* 2007; 117:978–88.
- 102 Chen G, Korfhagen TR, Xu Y *et al.* SPDEF is required for mouse pulmonary goblet cell differentiation and regulates a network of genes associated with mucus production. *J Clin Invest* 2009; 119:2914–24.
- 103 Song KS, Lee WJ, Chung KC *et al.* Interleukin-1 β and tumor necrosis factor- α induce MUC5AC overexpression through a mechanism involving ERK/p38 mitogen-activated

- protein kinases-MSK1-CREB activation in human airway epithelial cells. *J Biol Chem* 2003; **278**:23243–50.
- 104 Fujisawa T, Velichko S, Thai P, Hung LY, Huang F, Wu R. Regulation of airway MUC5AC expression by IL-1 beta and IL-17A; the NF-kappa B Paradigm. *J Immunol* 2009; **183**:6236–43.
 - 105 Shao MXG, Nakanaga T, Nadel JA. Cigarette smoke induces MUC5AC mucin overproduction via tumor necrosis factor-alpha-converting enzyme in human airway epithelial (NCI-H292) cells. *Am J Physiol Lung Cell Mol Physiol* 2004; **287**:L420–7.
 - 106 Takeyama K, Jung B, Shim JJ *et al.* Activation of epidermal growth factor receptors is responsible for mucin synthesis induced by cigarette smoke. *Am J Physiol Lung Cell Mol Physiol* 2001; **280**:L165–72.
 - 107 Parker D, Prince A. Innate immunity in the respiratory epithelium. *Am J Respir Cell Mol Biol* 2011; **45**:189–201.
 - 108 Rahman I, Biswas SK, Kode A. Oxidant and antioxidant balance in the airways and airway diseases. *Eur J Pharmacol* 2006; **533**:222–39.
 - 109 Kelly FJ. Oxidative stress: its role in air pollution and adverse health effects. *Occup Environ Med* 2003; **60**:612–6.
 - 110 Schwandner R, Dziarski R, Wesche H, Rothe M, Kirschning CJ. Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. *J Biol Chem* 1999; **274**:17406–9.
 - 111 Yoshimura A, Lien E, Ingalls RR, Tuomanen E, Dziarski R, Golenbock D. Cutting edge: recognition of gram-positive bacterial cell wall components by the innate immune system occurs via toll-like receptor 2. *J Immunol* 1999; **163**:1–5.
 - 112 Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-kappa B by Toll-like receptor 3. *Nature* 2001; **413**:732–8.
 - 113 Hoshino K, Takeuchi O, Kawai T *et al.* Cutting edge: toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. *J Immunol* 1999; **162**:3749–52.
 - 114 Diebold SS, Kaisho T, Hemmi H, Akira S, Sousa CRE. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science* 2004; **303**:1529–31.
 - 115 Akira S, Takeda K. Toll-like receptor signalling. *Nat Rev Immunol* 2004; **4**:499–511.
 - 116 Yoneyama M, Kikuchi M, Natsukawa T *et al.* The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat Immunol* 2004; **5**:730–7.
 - 117 Adams MN, Ramachandran R, Yau MK *et al.* Structure, function and pathophysiology of protease activated receptors. *Pharmacol Therapeut* 2011; **130**:248–82.
 - 118 Cambi A, Koopman M, Figdor CG. How C-type lectins detect pathogens. *Cell Microbiol* 2005; **7**:481–8.
 - 119 Uehara A, Fujimoto Y, Fukase K, Takada H. Various human epithelial cells express functional Toll-like receptors, NOD1 and NOD2 to produce antimicrobial peptides, but not proinflammatory cytokines. *Mol Immunol* 2007; **44**:3100–11.
 - 120 Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. *J Leukocyte Biol* 2007; **81**:1–5.
 - 121 Kono H, Rock KL. How dying cells alert the immune system to danger. *Nat Rev Immunol* 2008; **8**:279–89.
 - 122 Willart MAM, Lambrecht BN. The danger within: endogenous danger signals, atopy and asthma. *Clin Exp Allergy* 2009; **39**:12–9.
 - 123 Liew FY, Pitman NI, McInnes IB. Disease-associated functions of IL-33: the new kid in the IL-1 family. *Nat Rev Immunol* 2010; **10**:103–10.
 - 124 Kouzaki H, Iijima K, Kobayashi T, O'Grady SM, Kita H. The danger signal, extracellular ATP, is a sensor for an airborne allergen and triggers IL-33 release and innate Th2-type responses. *J Immunol* 2011; **186**:4375–87.
 - 125 Idzko M, Hammad H, van Nimwegen M *et al.* Extracellular ATP triggers and maintains asthmatic airway inflammation by activating dendritic cells. *Nat Med* 2007; **13**:913–9.
 - 126 Aikawa T, Shimura S, Sasaki H, Ebina M, Takishima T. Marked goblet cell hyperplasia with mucus accumulation in the airways of patients who died of severe acute asthma attack. *Chest* 1992; **101**:916–21.
 - 127 Kuyper LM, Pare PD, Hogg JC *et al.* Characterization of airway plugging in fatal asthma. *Am J Med* 2003; **115**:6–11.
 - 128 Shimura S, Andoh Y, Haraguchi M, Shirato K. Continuity of airway goblet cells and intraluminal mucus in the airways of patients with bronchial asthma. *Eur Respir J* 1996; **9**:1395–401.
 - 129 Pohunek P, Warner JO, Turzikova J, Kudrman J, Roche WR. Markers of eosinophilic inflammation and tissue re-modelling in children before clinically diagnosed bronchial asthma. *Pediatr Allergy Immunol* 2005; **16**:43–51.
 - 130 Saglani S, Payne DN, Zhu J *et al.* Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. *Am J Respir Crit Care Med* 2007; **176**:858–64.
 - 131 Fedorov IA, Wilson SJ, Davies DE, Holgate ST. Epithelial stress and structural remodelling in childhood asthma. *Thorax* 2005; **60**:389–94.
 - 132 Baraldo S, Turato G, Bazzan E *et al.* Noneosinophilic asthma in children: relation with airway remodelling. *Eur Respir J* 2011; **38**:575–83.
 - 133 Proud D, Leigh R. Epithelial cells and airway diseases. *Immunol Rev* 2011; **242**:186–204.
 - 134 Grainge CL, Lau LCK, Ward JA *et al.* Effect of bronchoconstriction on airway remodeling in asthma. *N Engl J Med* 2011; **364**:2006–15.
 - 135 Bucchieri F, Puddicombe SM, Lordan JL *et al.* Asthmatic bronchial epithelium is more susceptible to oxidant-induced apoptosis. *Am J Respir Cell Mol Biol* 2002; **27**:179–85.
 - 136 Leino MS, Loxham M, Blume C *et al.* Barrier disrupting effects of alternaria alternata extract on bronchial epithelium from asthmatic donors. *PLoS ONE* 2013; **8**:e71278.
 - 137 Hackett TL, Singhera GK, Shaheen F *et al.* Intrinsic phenotypic differences of asthmatic epithelium and its inflammatory responses to respiratory syncytial virus and air pollution. *Am J Respir Cell Mol Biol* 2011; **45**:1090–100.
 - 138 Wark PA, Johnston SL, Bucchieri F *et al.* Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med* 2005; **201**:937–47.
 - 139 Roth HM, Wadsworth SJ, Kahn M, Knight DA. The airway epithelium in

- asthma: developmental issues that scar the airways for life? *Pulm Pharmacol Ther* 2012; 25:420–6.
- 140 Davies DE. The role of the epithelium in airway remodeling in asthma. *Proc Am Thorac Soc* 2009; 6:678–82.
 - 141 Wilson JW, Li X. The measurement of reticular basement membrane and submucosal collagen in the asthmatic airway. *Clin Exp Allergy* 1997; 27:363–71.
 - 142 Araujo BB, Dolhnikoff M, Silva LFF *et al.* Extracellular matrix components and regulators in the airway smooth muscle in asthma. *Eur Respir J* 2008; 32:61–9.
 - 143 Faux SP, Tai T, Thorne D, Xu Y, Breheny D, Gaca M. The role of oxidative stress in the biological responses of lung epithelial cells to cigarette smoke. *Biomarkers* 2009; 14 (Suppl 1):90–6.
 - 144 Li N, Xia T, Nel AE. The role of oxidative stress in ambient particulate matter-induced lung diseases and its implications in the toxicity of engineered nanoparticles. *Free Radic Biol Med* 2008; 44:1689–99.
 - 145 Nakayama Wong LS, Aung HH, Lame MW, Wegesser TC, Wilson DW. Fine particulate matter from urban ambient and wildfire sources from California's San Joaquin Valley initiate differential inflammatory, oxidative stress, and xenobiotic responses in human bronchial epithelial cells. *Toxicol In Vitro* 2011; 25:1895–905.
 - 146 Jaspers I, Ciencewicz JM, Zhang W *et al.* Diesel exhaust enhances influenza virus infections in respiratory epithelial cells. *Toxicol Sci* 2005; 85:990–1002.
 - 147 Hosakote YM, Liu T, Castro SM, Garofalo RP, Casola A. Respiratory syncytial virus induces oxidative stress by modulating antioxidant enzymes. *Am J Respir Cell Mol Biol* 2009; 41:348–57.
 - 148 Papi A, Contoli M, Gasparini P *et al.* Role of xanthine oxidase activation and reduced glutathione depletion in rhinovirus induction of inflammation in respiratory epithelial cells. *J Biol Chem* 2008; 283:28595–606.
 - 149 Fitzpatrick AM, Jones DP, Brown LA. Glutathione redox control of asthma: from molecular mechanisms to therapeutic opportunities. *Antioxid Redox Signal* 2012; 17:375–408.
 - 150 Piacentini S, Polimanti R, Simonelli I *et al.* Glutathione S-transferase polymorphisms, asthma susceptibility and confounding variables: a meta-analysis. *Mol Biol Rep* 2013; 40:3299–313.
 - 151 Wu W, Peden DB, McConnell R, Fruin S, Diaz-Sanchez D. Glutathione-S-transferase M1 regulation of diesel exhaust particle-induced pro-inflammatory mediator expression in normal human bronchial epithelial cells. *Part Fibre Toxicol* 2012; 9:31.
 - 152 Li N, Nel AE. Role of the Nrf2-mediated signaling pathway as a negative regulator of inflammation: implications for the impact of particulate pollutants on asthma. *Antioxid Redox Signal* 2006; 8:88–98.
 - 153 Mercado N, Thimmulappa R, Thomas CM *et al.* Decreased histone deacetylase 2 impairs Nrf2 activation by oxidative stress. *Biochem Biophys Res Commun* 2011; 406:292–8.
 - 154 Ito K, Hanazawa T, Tomita K, Barnes PJ, Adcock IM. Oxidative stress reduces histone deacetylase 2 activity and enhances IL-8 gene expression: role of tyrosine nitration. *Biochem Biophys Res Commun* 2004; 315:240–5.
 - 155 Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science* 2011; 334:1081–6.
 - 156 Garg AD, Kaczmarek A, Krysko O, Vandenabeele P, Krysko DV, Agostinis P. ER stress-induced inflammation: does it aid or impede disease progression? *Trends Mol Med* 2012; 18:589–98.
 - 157 Tanjore H, Lawson WE, Blackwell TS. Endoplasmic reticulum stress as a pro-fibrotic stimulus. *Biochim Biophys Acta* 2013; 1832:940–7.
 - 158 Wei J, Rahman S, Ayaub EA, Dickhout JG, Ask K. Protein misfolding and endoplasmic reticulum stress in chronic lung disease. *Chest* 2013; 143:1098–105.
 - 159 Miller M, Tam AB, Cho JY *et al.* ORMDL3 is an inducible lung epithelial gene regulating metalloproteases, chemokines, OAS, and ATF6. *Proc Natl Acad Sci USA* 2012; 109:16648–53.
 - 160 Schmidt LM, Belvisi MG, Bode KA *et al.* Bronchial epithelial cell-derived prostaglandin E2 dampens the reactivity of dendritic cells. *J Immunol* 2011; 186:2095–105.
 - 161 Saffholm J, Dahlen SE, Delin I *et al.* PGE2 maintains the tone of the guinea pig trachea through a balance between activation of contractile EP1 receptors and relaxant EP2 receptors. *Br J Pharmacol* 2013; 168:794–806.
 - 162 Garrison G, Huang SK, Okunishi K *et al.* Reversal of myofibroblast differentiation by prostaglandin E2. *Am J Respir Cell Mol Biol* 2013; 48:550–8.
 - 163 Sastre B, del Pozo V. Role of PGE2 in asthma and nonasthmatic eosinophilic bronchitis. *Mediators Inflamm* 2012; 2012:645383.
 - 164 Romano M. Lipoxin and aspirin-triggered lipoxins. *ScientificWorldJournal* 2010; 10:1048–64.
 - 165 Levy BD. Resolvin D1 and resolvin E1 promote the resolution of allergic airway inflammation via shared and distinct molecular counter-regulatory pathways. *Front Immunol* 2012; 3:390.
 - 166 Planaguma A, Levy BD. Uncontrolled airway inflammation in lung disease represents a defect in counter-regulatory signaling. *Future Lipidol* 2008; 3:697–704.
 - 167 Hallstrand TS, Lai Y, Henderson WR Jr, Altemeier WA, Gelb MH. Epithelial regulation of eicosanoid production in asthma. *Pulm Pharmacol Ther* 2012; 25:432–7.
 - 168 Hallstrand TS, Henderson WR. An update on the role of leukotrienes in asthma. *Curr Opin Allergy Clin Immunol* 2010; 10:60–6.
 - 169 Hallstrand TS, Wurfel MM, Lai Y *et al.* Transglutaminase 2, a novel regulator of eicosanoid production in asthma revealed by genome-wide expression profiling of distinct asthma phenotypes. *PLoS ONE* 2010; 5:e8583.
 - 170 Lai Y, Oslund RC, Bollinger JG *et al.* Eosinophil cysteinyl leukotriene synthesis mediated by exogenous secreted phospholipase A2 group X. *J Biol Chem* 2010; 285:41491–500.
 - 171 Bartemes KR, Kita H. Dynamic role of epithelium-derived cytokines in asthma. *Clin Immunol* 2012; 143:222–35.
 - 172 Ingram JL, Kraft M. IL-13 in asthma and allergic disease: asthma phenotypes and targeted therapies. *J Allergy Clin Immunol* 2012; 130:829–42; quiz 43–4.
 - 173 Maes T, Joos GF, Brusselle GG. Targeting interleukin-4 in asthma: lost in translation? *Am J Respir Cell Mol Biol* 2012; 47:261–70.

- 174 Swindle EJ, Collins JE, Davies DE. Breakdown in epithelial barrier function in patients with asthma: identification of novel therapeutic approaches. *J Allergy Clin Immunol* 2009; 124:23–34.
- 175 Blume C, Swindle EJ, Dennison P *et al.* Barrier responses of human bronchial epithelial cells to grass pollen exposure. *Eur Respir J* 2013; 42:87–97.
- 176 Coleman JM, Naik C, Holguin F *et al.* Epithelial eotaxin-2 and eotaxin-3 expression: relation to asthma severity, luminal eosinophilia and age at onset. *Thorax* 2012; 67:1061–6.
- 177 Uller L, Leino M, Bedke N *et al.* Double-stranded RNA induces disproportionate expression of thymic stromal lymphopoietin versus interferon-beta in bronchial epithelial cells from donors with asthma. *Thorax* 2010; 65:626–32.
- 178 Freishtat RJ, Watson AM, Benton AS *et al.* Asthmatic airway epithelium is intrinsically inflammatory and mitotically dyssynchronous. *Am J Respir Cell Mol Biol* 2011; 44:863–9.
- 179 Yang IV, Schwartz DA. Epigenetic mechanisms and the development of asthma. *J Allergy Clin Immunol* 2012; 130:1243–55.
- 180 Zijlstra GJ, Ten Hacken NH, Hoffmann RF, van Oosterhout AJ, Heijink IH. Interleukin-17A induces glucocorticoid insensitivity in human bronchial epithelial cells. *Eur Respir J* 2012; 39:439–45.
- 181 Li LB, Leung DY, Martin RJ, Goleva E. Inhibition of histone deacetylase 2 expression by elevated glucocorticoid receptor beta in steroid-resistant asthma. *Am J Respir Crit Care Med* 2010; 182:877–83.
- 182 Butler CA, McQuaid S, Taggart CC *et al.* Glucocorticoid receptor beta and histone deacetylase 1 and 2 expression in the airways of severe asthma. *Thorax* 2012; 67:392–8.
- 183 Baraldo S, Contoli M, Bazzan E *et al.* Deficient antiviral immune responses in childhood: distinct roles of atopy and asthma. *J Allergy Clin Immunol* 2012; 130:1307–14.
- 184 Contoli M, Message SD, Laza-Stanca V *et al.* Role of deficient type III interferon-lambda production in asthma exacerbations. *Nat Med* 2006; 12:1023–6.
- 185 Bochkov YA, Hanson KM, Keles S, Brockman-Schneider RA, Jarjour NN, Gern JE. Rhinovirus-induced modulation of gene expression in bronchial epithelial cells from subjects with asthma. *Mucosal Immunol* 2010; 3:69–80.
- 186 Lopez-Souza N, Favoreto S, Wong H *et al.* In vitro susceptibility to rhinovirus infection is greater for bronchial than for nasal airway epithelial cells in human subjects. *J Allergy Clin Immunol* 2009; 123:1384–90.
- 187 Schoggins JW, Rice CM. Interferon-stimulated genes and their antiviral effector functions. *Curr Opin Virol* 2011; 1:519–25.
- 188 Corne JM, Marshall C, Smith S *et al.* Frequency, severity, and duration of rhinovirus infections in asthmatic and non-asthmatic individuals: a longitudinal cohort study. *Lancet* 2002; 359:831–4.
- 189 Holtzman MJ. Asthma as a chronic disease of the innate and adaptive immune systems responding to viruses and allergens. *J Clin Invest* 2012; 122:2741–8.
- 190 Gavala ML, Bertics PJ, Gern JE. Rhinoviruses, allergic inflammation, and asthma. *Immunol Rev* 2011; 242:69–90.
- 191 Halwani R, Al-Muhsen S, Al-Jahdali H, Hamid Q. Role of transforming growth factor-beta in airway remodeling in asthma. *Am J Respir Cell Mol Biol* 2011; 44:127–33.
- 192 Tatler AL, Jenkins G. TGF-beta activation and lung fibrosis. *Proc Am Thorac Soc* 2012; 9:130–6.
- 193 Lopez-Guisa JM, Powers C, File D, Cochrane E, Jimenez N, Debley JS. Airway epithelial cells from asthmatic children differentially express proremodeling factors. *J Allergy Clin Immunol* 2012; 129:990–7.e6.
- 194 Boxall C, Holgate ST, Davies DE. The contribution of transforming growth factor-beta and epidermal growth factor signalling to airway remodelling in chronic asthma. *Eur Respir J* 2006; 27:208–29.
- 195 Doerner AM, Zuraw BL. TGF-beta1 induced epithelial to mesenchymal transition (EMT) in human bronchial epithelial cells is enhanced by IL-1beta but not abrogated by corticosteroids. *Respir Res* 2009; 10:100.
- 196 Bedke N, Sammut D, Green B *et al.* Transforming growth factor-beta promotes rhinovirus replication in bronchial epithelial cells by suppressing the innate immune response. *PLoS ONE* 2012; 7:e44580.
- 197 Skevaki CL, Psarras S, Volonaki E *et al.* Rhinovirus-induced basic fibroblast growth factor release mediates airway remodeling features. *Clin Transl Allergy* 2012; 2:14.
- 198 Leigh R, Oyelusi W, Wiehler S *et al.* Human rhinovirus infection enhances airway epithelial cell production of growth factors involved in airway remodeling. *J Allergy Clin Immunol* 2008; 121:1238–45.
- 199 Meyer N, Akdis CA. Vascular endothelial growth factor as a key inducer of angiogenesis in the asthmatic airways. *Curr Allergy Asthma Rep* 2013; 13:1–9.
- 200 Burgel PR, Nadel JA. Epidermal growth factor receptor-mediated innate immune responses and their roles in airway diseases. *Eur Respir J* 2008; 32:1068–81.
- 201 Royce SG, Cheng V, Samuel CS, Tang ML. The regulation of fibrosis in airway remodeling in asthma. *Mol Cell Endocrinol* 2012; 351:167–75.
- 202 Loffek S, Schilling O, Franzke CW. Series “matrix metalloproteinases in lung health and disease”: biological role of matrix metalloproteinases: a critical balance. *Eur Respir J* 2011; 38:191–208.
- 203 Tacon CE, Wiehler S, Holden NS, Newton R, Proud D, Leigh R. Human rhinovirus infection up-regulates MMP-9 production in airway epithelial cells via NF- κ B. *Am J Respir Cell Mol Biol* 2010; 43:201–9.
- 204 Sidhu SS, Yuan S, Innes AL *et al.* Roles of epithelial cell-derived periostin in TGF-beta activation, collagen production, and collagen gel elasticity in asthma. *Proc Natl Acad Sci USA* 2010; 107:14170–5.
- 205 Waldeck B. β -adrenoceptor agonists and asthma – 100 years of development. *Eur J Pharmacol* 2002; 445:1–12.
- 206 Barnes PJ. Efficacy of inhaled corticosteroids in asthma. *J Allergy Clin Immunol* 1998; 102:531–8.
- 207 Bousquet J, Cabrera P, Berkman N *et al.* The effect of treatment with omalizumab, an anti-IgE antibody, on asthma exacerbations and emergency medical visits in patients with severe

- persistent asthma. *Allergy* 2005; **60**:302–8.
- 208 Walker S, Monteil M, Phelan K, Lasserer TJ, Walters EH. Anti-IgE for chronic asthma in adults and children. *Cochrane Database Syst Rev* 2006; (2):CD003559.
- 209 Holgate ST. Trials and tribulations in identifying new biologic treatments for asthma. *Trends Immunol* 2012; **33**:238–46.
- 210 Bates JHT, Rincon M, Irvin CG. Animal models of asthma. *Am J Physiol Lung Cell Mol Physiol* 2009; **297**:L401–10.
- 211 Zosky GR, Sly PD. Animal models of asthma. *Clin Exp Allergy* 2007; **37**:973–88.
- 212 Blume C, Davies DE. In vitro and ex vivo models of human asthma. *Eur J Pharm Biopharm* 2013; **84**:394–400.
- 213 Allan K, Devereux G. Diet and asthma: nutrition implications from prevention to treatment. *J Am Diet Assoc* 2011; **111**:258–68.
- 214 He X, Wang L, Szklarz G, Bi Y, Ma Q. Resveratrol inhibits paraquat-induced oxidative stress and fibrogenic response by activating the nuclear factor erythroid 2-related factor 2 pathway. *J Pharmacol Exp Ther* 2012; **342**:81–90.
- 215 Zhang HQ, Shih A, Rinna A, Forman HJ. Exacerbation of tobacco smoke mediated apoptosis by resveratrol: an unexpected consequence of its antioxidant action. *Int J Biochem Cell B* 2011; **43**:1059–64.
- 216 Kode A, Rajendrasozhan S, Caito S, Yang SR, Megson IL, Rahman I. Resveratrol induces glutathione synthesis by activation of Nrf2 and protects against cigarette smoke-mediated oxidative stress in human lung epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2008; **294**:L478–88.
- 217 Hofmann B, Steinhilber D. 5-Lipoxygenase inhibitors: a review of recent patents (2010–2012). *Expert Opin Ther Pat* 2013; **23**:895–909.
- 218 Malhotra S, Deshmukh SS, Dastidar SG. COX inhibitors for airway inflammation. *Expert Opin Ther Targets* 2012; **16**:195–207.
- 219 Benyahia C, Gomez I, Kanyinda L et al. PGE(2) receptor (EP(4)) agonists: potent dilators of human bronchi and future asthma therapy? *Pulm Pharmacol Ther* 2012; **25**:115–8.
- 220 Hisada T, Ishizuka T, Aoki H, Mori M. Resolvin E1 as a novel agent for the treatment of asthma. *Expert Opin Ther Targets* 2009; **13**:513–22.
- 221 Lee CH. Resolvins as new fascinating drug candidates for inflammatory diseases. *Arch Pharmacol Res* 2012; **35**:3–7.
- 222 Grumbach Y, Quynh NV, Chiron R, Urbach V. LXA4 stimulates ZO-1 expression and transepithelial electrical resistance in human airway epithelial (16HBE14o-) cells. *Am J Physiol Lung Cell Mol Physiol* 2009; **296**:L101–8.
- 223 Corren J, Lemanske RF, Hanania NA et al. Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011; **365**:1088–98.
- 224 Slager RE, Otulana BA, Hawkins GA et al. IL-4 receptor polymorphisms predict reduction in asthma exacerbations during response to an anti-IL-4 receptor alpha antagonist. *J Allergy Clin Immunol* 2012; **130**:516–22.e4.
- 225 Wenzel S, Ford L, Pearlman D et al. Dupilumab in persistent asthma with elevated eosinophil levels. *N Engl J Med* 2013; **368**:2455–66.
- 226 Liu Y, Zhang S, Li DW, Jiang SJ. Efficacy of anti-interleukin-5 therapy with mepolizumab in patients with asthma: a meta-analysis of randomized placebo-controlled trials. *PLoS ONE* 2013; **8**:e59872.
- 227 LaFemina MJ, Rokkam D, Chandrasena A et al. Keratinocyte growth factor enhances barrier function without altering claudin expression in primary alveolar epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2010; **299**:L724–34.
- 228 Cakebread JA, Xu Y, Grainge C et al. Exogenous IFN-beta has antiviral and anti-inflammatory properties in primary bronchial epithelial cells from asthmatic subjects exposed to rhinovirus. *J Allergy Clin Immunol* 2011; **127**:1148–54.e9.