Role of air pollutants in airway epithelial barrier dysfunction in asthma and COPD

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Abstract

Chronic exposure to environmental pollutants is a major contributor to the development and progression of obstructive airway diseases, including asthma and COPD. Understanding the mechanisms underlying the development of obstructive lung diseases upon exposure to inhaled pollutants will lead to novel insights into the pathogenesis, prevention and treatment of these diseases. The respiratory epithelial lining forms a robust physicochemical barrier protecting the body from inhaled toxic particles and pathogens. Inhalation of airborne particles and gases may impair airway epithelial barrier function and subsequently lead to exaggerated inflammatory responses and airway remodelling, which are key features of asthma and COPD. In addition, air pollutant-induced airway epithelial barrier dysfunction may increase susceptibility to respiratory infections, thereby increasing the risk of exacerbations and thus triggering further inflammation. In this review, we discuss the molecular and immunological mechanisms involved in physical barrier disruption induced by major airborne pollutants and outline their implications in the pathogenesis of asthma and COPD. We further discuss the link between these pollutants and changes in the lung microbiome as a potential factor for aggravating airway diseases. Understanding these mechanisms may lead to identification of novel targets for therapeutic intervention to restore airway epithelial integrity in asthma and COPD.

Introduction

Obstructive airway diseases, including COPD and asthma, are respiratory diseases characterised by airflow limitation, chronic airway inflammation and progressive decline in lung function [1]. By 2015, more than half a billion individuals were diagnosed with asthma or COPD worldwide [2]. Although asthma cases account for up to two-thirds of these cases, the mortality rate in COPD is twice that of asthma, which is in part explained by the fact that COPD is mostly diagnosed among elderly patients [2]. Environmental exposures, including air pollutants from burning biomass and fossil fuels, dust, nanoparticles that are emitted by chemical industries, microplastics in textiles, emissions from large farms, and detergents used for laundry, expose a large population to health risks both in developed and developing countries [3]. These environmental factors in combination with genetic susceptibility factors constitute the major risk factors for chronic airway diseases [4]. In addition, such pollutants can worsen the symptoms and exacerbate asthma and COPD, as demonstrated by the observation that each year many patients are being

admitted to hospital at times of reduced air quality during both extremely high and low temperature days [5–7]. These exposures may increase susceptibility to airway infections with viral and bacterial pathogens, which are considered to be the major driving factors for exacerbations [8]. The airway epithelium functions as a mechanical and immunologic barrier by its physical barrier function, removal of noxious agents by employing mucociliary machinery, metabolism of pollutants and production of antimicrobial and immune mediators, collectively serving to protect the gas exchange unit, *i.e.* the alveoli, as well as the submucosal layers from the inhaled environment [9]. Due to its anatomical location as the first point in the respiratory system encountering these exposures, it is more susceptible to damage than the alveoli. Indeed, there is increasing evidence to suggest that COPD cases that are linked to exposure to the emissions released by burning biomass fuels show a distinct phenotype with more prominent airway disease and less alveolar damage (emphysema) [10, 11]. Impaired airway epithelial barrier function is involved in the complex pathogenesis of both asthma and COPD, both of which are obstructive airway diseases [9, 12]. Many air pollutants inflict epithelial damage by inducing oxidative stress [13], propagating barrier dysfunction, pro-inflammatory responses and remodelling. Impaired epithelial repair mechanisms may lead to persistent barrier disruption [9]. Prolonged exposure to many of the inhaled air pollutants directly or indirectly causes airway epithelial barrier dysfunction [12], which may subsequently facilitate colonisation and invasion of respiratory pathogens, and thus may contribute to the development and progression of COPD and asthma.

In this review, we provide a comprehensive, up-to-date overview on the mechanisms of air pollutant-induced airway epithelial barrier dysfunction and outline how this contributes to airway epithelial remodelling, epithelial innate immune dysfunction and infections in asthma and COPD. Furthermore, by discussing potential therapeutics that have been experimentally found to resolve airway epithelial barrier dysfunction induced by air pollutants in different airway disease models, we aim to stress the clinical implications of this novel insight.

Sources, compositions and respiratory hazards of air pollutants

Air pollutants are generally categorised based on their nature (gas or particles), sources (indoor and outdoor) and size (ultrafine, fine and coarse) [14]. Air pollutants with either indoor or outdoor air origin are a mixture of gas and solid-phase varied-size particles but with different origins [14]. Indoor air pollution mainly originates from stoves, biologic materials (such as mould), microplastics and household dust [15], whereas outdoor pollutants originate from vehicles and industrial (urban)/agricultural activities (rural) [14]. Outdoor particulate matter (PM) is a heterogenous mixture that can consist of airborne dust and heavy metals as well as nanoparticles emitted by vehicles, wildfire smoke, volcano eruptions and chemical industries [16]. The majority of outdoor urban PM originates from incomplete burning of fossil fuels by transport vehicles in cities [17].

PM ranges from ultrafine particles with diameters $\leqslant$0.1 µm or $PM_{0.1}$ (*e.g.* nanoparticles), fine particles or $PM_{2.5}$ $\leqslant$2.5 µm (*e.g.* vehicle exhaust) and coarse particles or $PM_{10–2.5}$ 2.5–10 µm (*e.g.* dust) [16]. Fine PM is particularly harmful to inhale as it travels deeper into the small airways than coarse PM [16]. Indeed, inhalation of fine PM has been associated with the progression of asthma and COPD [18–20]. In addition to black carbon, $PM_{2.5}$ carries chemical components such as sulfate, nitrate, ammonium and silicon, as well as gas phase polyaromatic hydrocarbon (PAH), mainly originating from either industrial sources such as chemical industries or burning of fossil fuels as well as emissions from large farms [14]. Apart from PAH, other gas phase pollutants such as ozone, sulphur dioxide (SO_2), nitrogen oxides (NO_x), carbon monoxide (CO) and methane appear separately from $PM_{2.5}$ in ambient air pollution [14]. Ozone is formed as a by-product of a reaction between volatile organic compounds and NO_x originated from outdoor air pollution in the presence of sunlight [17] and PAH originates from burning of organic materials such as oil, woods and coal [14].

The average concentrations of $PM_{0.1}$, $PM_{2.5}$, $PM_{10–2.5}$ in outdoor air have been reported to be affected by season and have been calculated as 16–58, 27–58 and 20–42 $\mu g \cdot m^{-3}$, respectively [21, 22]. Recent World Health Organization (WHO) air quality guidelines recommend the daily levels of $PM_{2.5}$ and $PM_{10–2.5}$ in the air to be kept lower than 15 $\mu g \cdot m^{-3}$ and 45 $\mu g \cdot m^{-3}$, respectively [23]. A 10 $\mu g \cdot m^{-3}$ increase in $PM_{2.5}$ and $PM_{10–2.5}$ levels was associated with higher mortality and prevalence of respiratory diseases [24]. Furthermore, short- or long-term exposure to air pollutants is closely linked to respiratory diseases such as asthma and COPD [14]. Increased levels of outdoor pollution, in particular NO_2, $PM_{2.5}$ and black carbon levels, have also been associated with the onset and progression of childhood asthma [18]. Additionally, a recent large cross-sectional study revealed a strong association between increased levels of $PM_{2.5}$ and NO_2 and decreased lung function and prevalence of COPD [19]. Volcano ash released upon volcanic eruptions has been considered as an air pollutant with a potential hazardous impact on respiratory function [25]. Volcano ash is composed of many known air pollutants, including fine PM, iron, silica (SiO_2) and SO_2.

It has been shown that volcanic emission exposure, specifically SO_2, is associated with increased hospitalisation in patients with respiratory diseases, particularly in those with COPD and asthma [26].

Airway epithelial barrier function in health and obstructive lung diseases

The pseudostratified human airway epithelium is composed of several subsets of cells, including secretory goblet and club cells, ciliated cells, basal cells and more rare cell types such as tuft cells, neuroendocrine cells and recently identified ionocytes, each responsible for a specific function (figure 1a) [27]. Mucus produced by goblet cells trap and neutralise noxious particles that can subsequently be cleared by ciliary movement of the ciliated cells. Basal cells can regenerate these secretory subsets and ciliated cells [27]. Airway epithelial cells (AECs) protect the submucosal layer against noxious particles, allergens and respiratory pathogens, by forming both physical, mucosal and innate immune barriers [9]. The physical airway epithelial barriers include junctions between the adjacent cells. At the apical side of the cells, tight junctions (TJs) including zona occludens (ZO)-1, ZO-2, claudin family members, the junctional adhesion molecule (JAM) family, occludin, tricellulin and marvelD3, stabilise the barrier. At the basolateral side of TJs, adherens junctions (AJs), comprising the transmembrane protein E-cadherin, attach the cells to each other [12]. AJs and TJs are linked together *via* ZOs and the cell polarity proteins Par complex (Par1–6) [9] and to the cytoskeleton machinery through scaffolding proteins, *e.g.* actin filaments, cingulin and β-catenin [12]. TJs serve as ionic gates which chiefly regulate the passage of ions and small peptides between the epithelial cells, yet function as fences which compartmentalise apical and basal parts of the cell establishing cell polarity [12]. In contrast, AJs attach the adjacent AECs at the basolateral side, providing a robust attachment that facilitates the formation of other junctional complexes. Together AJs and TJs keep the sub-epithelium protected from penetration of noxious particles and respiratory pathogens [12]. Although both AJs and TJs are expressed by all subsets of AECs, specific subsets may be more susceptible to barrier dysfunction resulting from air pollutants. For instance, since ciliated cells are located on the luminal surfaces, and are in direct contact with the inhaled particles, they are likely to be more susceptible to damaging insults than basal cells [28].

Loss of epithelial cell–cell contact has been observed in several obstructive airway diseases, including asthma and COPD [9, 12, 29]. Of note, decreased expression of several TJs and AJs, including E-cadherin, β-catenin, occludin and ZO-1 in the airway epithelium of patients with asthma and patients with COPD was reported to be accompanied by diminished ciliary function [9, 12]. Airway epithelial damage and loss of barrier function may not only lead to increased antigen uptake and antigen-presenting immune cells such as dendritic cells, but also to increased pro-inflammatory activity of the epithelium, leading to secretion of danger-associated molecular patterns (DAMPs), cytokines and chemokines and recruitment of innate and adaptive immune cells (figure 1b) [9]. Further, loss of barrier function is accompanied by impaired antimicrobial activity [30]. Meanwhile, other changes in epithelial function are observed in obstructive lung diseases, such as mucociliary dysfunction with the loss of cilia, impaired ciliary beating and mucus hypersecretion contributing to airway obstruction [9].

Molecular mechanisms involved in air pollutant-induced airway epithelial barrier dysfunction

Mechanisms of physical barrier disruption by particulate matter

Numerous studies showed that air pollutants, as one of the major risk factors for development of asthma and COPD, induce airway epithelial barrier disruption [31–35]. $PM_{2.5}$ present in *e.g.* traffic-related air pollution is among the most studied outdoor air pollutants affecting epithelial integrity [36]. In addition, atmospheric gases such as ozone, ammonia and SO_2 have been shown to induce airway epithelial barrier dysfunction [33, 34, 37–39]. Studies show that repetitive short sub-toxic exposures to soluble $PM_{2.5}$ induces airway epithelial barrier disruption by downregulation of TJs, E-cadherin, decline in transepithelial electrical resistance (TEER) and an increase in paracellular permeability *in vitro* [34, 35, 40]. Furthermore, diesel exhaust as one of the major sources of PM in cities was shown to decrease TEER and reduce the expression of ZO-1 and E-cadherin in AECs *in vitro* and *in vivo* [31, 32, 41]. An increase in reactive oxygen species (ROS) production following exposure to air pollutants may serve as a key mechanism in airway epithelial barrier dysfunction. This resulting increase in oxidative stress can originate either from direct free radical activity of components (*e.g.* metals in PM), from the activation of cellular ROS-generating systems such as nicotinamide adenine dinucleotide phosphate (NADPH) and dual oxidase, or by altering mitochondrial function [42]. Moreover, air pollutants can enhance ROS levels by suppressing lung antioxidant mechanisms. While oxidative stress responses usually lead to activation of the antioxidant machinery, such as superoxide dismutase (SOD), nuclear factor E2-related factor 2 (Nrf2) and antioxidant responsive element (ARE)-mediated transcriptional responses, PM reduced these mechanisms particularly at higher doses [43]. Furthermore, a decrease in SOD2 expression has been observed in the lungs of mice repeatedly exposed to high concentration of $PM_{2.5}$ prior to a haze period [44]. In addition, polymorphisms in antioxidant enzyme genes (*GSTM1*, *GSTP1*, *GSTT1* and *NQO1*) have been associated with higher

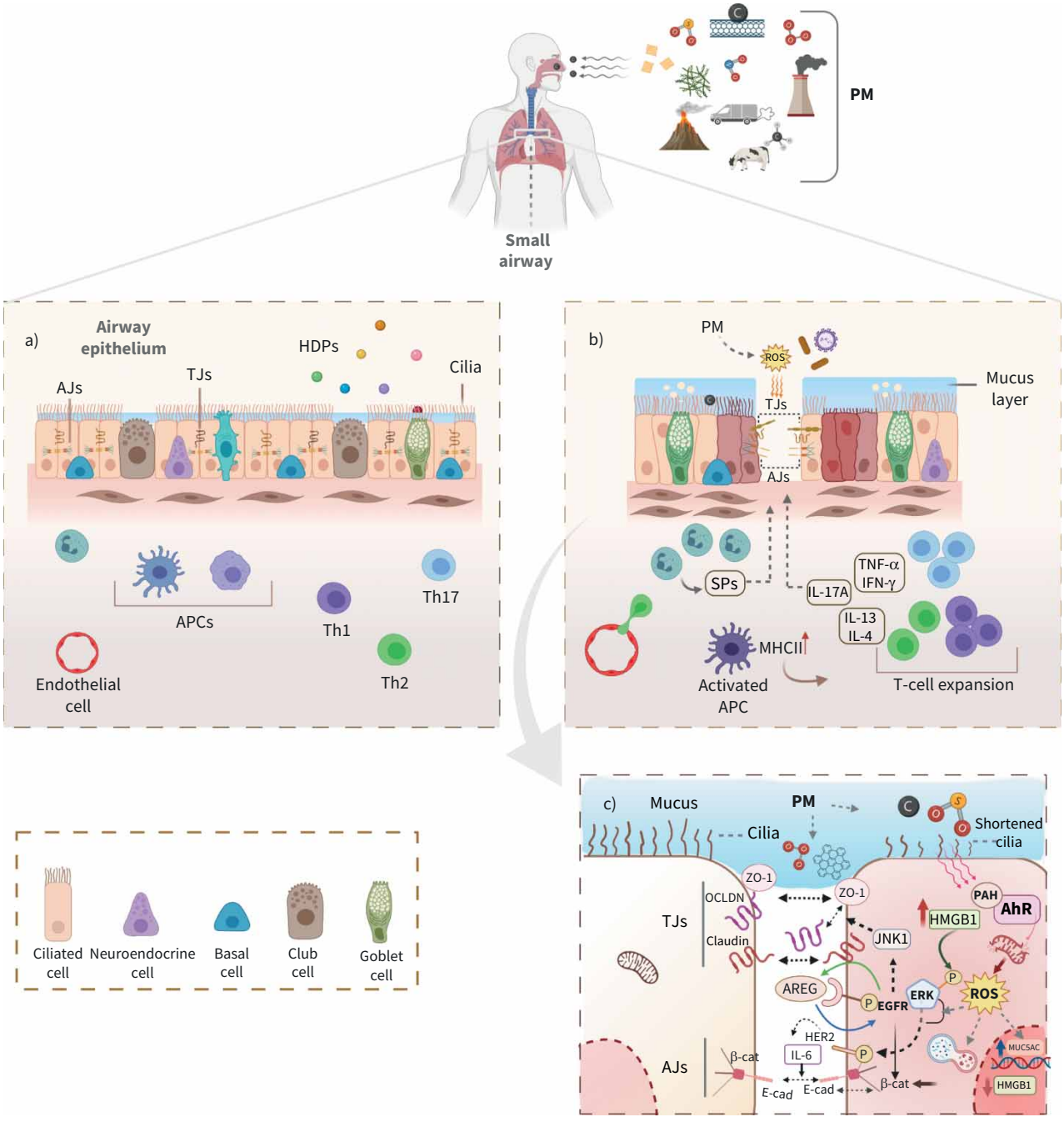

FIGURE 1 Inhalation of air pollutants promotes airway epithelial barrier dysfunction. a) Healthy airway epithelium is protected against pathogens by physical junctions between the adjacent cells and by releasing innate immune HDPs. b) By exposure to airborne PM, the TJs and AJs between the AECs, including ZO-1, occludin and E-cadherin, are disrupted. Furthermore, PM induces mucociliary dysfunction by reducing cilia number, loss of cilia as well as goblet cell metaplasia leading to increased mucus production. PM entering the airway submucosa is presented to adaptive immune cells by APCs. PM-induced increase in MHCII on APCs leads to expansion of adaptive immune cells and production of inflammatory cytokines that further inflict airway epithelial barrier dysfunction. PM exposure also induces submucosal accumulation of neutrophils which may lead to barrier dysfunction by secretion of SPs. c) PM-induced barrier dysfunction is in part triggered by AhR-mediated increase in ROS generation by mitochondria and subsequent activation of EGFR and ERK or ROS-mediated increase in cytoplasmic HMGB1 and ROS-mediated increase in autophagy in AECs. Activation of EGFR either by PM-induced increase in AREG or ROS leads to disassembly in AJs by disrupting E-cadherin/β-catenin, and increased TJs permeability by Rac1/JNK-mediated disruption in ZO-1 and occludin. Activation of ERK in turn triggers airway barrier dysfunction through HER2-mediated increase in IL-6. AEC: airway epithelial cell; AhR: aryl hydrocarbon receptor; AJ: adherens junction; APC: antigen-presenting cell; AREG: amphiregulin; β-cat: β-catenin; E-cad: E-cadherin; EGFR: epidermal growth factor receptor; ERK: extracellular signal-regulated kinase; HDP: host defence proteins and peptide; HER2: human epidermal receptor 2; HMGB1: high-mobility group box 1; IFN: interferon; IL: interleukin; JNK: c-jun N-terminal kinase; MHCII: major histocompatibility complex class II; MUC5AC: mucin 5AC; OCLDN: occludin; PAH: polyaromatic hydrocarbon; PM: particulate matter; ROS: reactive oxygen species; SP: serine protease; Th: T-helper cell; TJ: tight junction; TNF: tumour necrosis factor; ZO-1: zonula occludens-1. Figure partially created with BioRender.com.

susceptibility to lung function decline upon exposure to air pollutants, highlighting the role of redox imbalance in the development of respiratory disease upon exposure to air pollutants [45, 46].

In urban areas the fine and ultrafine PM organic carbon-derivative components such as PAH and quinones (*e.g.* benzene and naphthalene) are largely responsible for oxidative damage [47]. $PM_{2.5}$ and PAH induces ROS *via* the aryl hydrocarbon receptor and subsequent cytochrome p450 activation [48]. However, inorganic materials in $PM_{2.5}$ such as transition metals (iron, copper, silicon *etc*.) and nanoparticles as well as gas phase NO_x and ozone display free radical activity and may thus increase the oxidative burden [47]. In contrast, coarse particles are mainly composed of transition metals, which have been described to induce oxidative stress in the lung [49], either *via* direct free radical activity [47] or *via* increased lipid peroxidation in the lung [50].

Co-exposure to $PM_{10–2.5}$ and lipopolysaccharide attenuated E-cadherin and claudin1 in ALI-cultured AECs *in vitro* possibly *via* ROS-mediated activation of aryl hydrocarbon receptor (AhR) and through mitochondrial cytochrome p450 activation [51]. ROS-mediated activation of epidermal growth factor receptor (EGFR) and downstream extracellular signal-regulated kinase (ERK) signalling is one of the molecular mechanisms that has been implicated in airway epithelial barrier disruption by affecting both TJs and AJs [9]. While high E-cadherin expression limits EGFR activity, activation of EGFR leads to redistribution of E-cadherin and vice versa, by causing tyrosine phosphorylation of β-catenin through Src kinases [52], internalisation of E-cadherin and increased paracellular permeability by Rac1/c-jun N-terminal kinase (JNK)-mediated decrease in ZO-1 and occludin junctional localisation [53]. Of note, PM-induced EGFR activation in AECs was shown to induce airway epithelial barrier disruption in AECs [54, 55]. Non-toxic doses of whole $PM_{2.5}$, and organic extract of $PM_{2.5}$ mainly containing PAH, were shown to increase the release of the EGFR ligand amphiregulin over 24 h up to 48 h post-exposure in AECs *in vitro* [56, 57]. This increased release of EGFR ligands upon PM exposure was reported to be dependent on metalloproteinases such as metalloproteinase 17 (ADAM17) also known as (TNF-α)-converting enzyme (TACE) [58], which proteolytically cleaves the active form of amphiregulin. PM-induced ROS release has been implicated in the increase of amphiregulin release in AECs [55], which may subsequently lead to EGFR/ERK activation and airway epithelial barrier disruption by the destabilisation of junctional proteins. The activation of EGFR by PM was shown to lead to further release of amphiregulin in an ERK1-dependent manner [59]. Cytoplasmic release of high-mobility group box 1 (HMGB1), a chromatin-binding protein and DAMP released upon cellular damage, has also been shown to cause a disruption in TJs and AJs *via* activation of ERK in AECs *in vitro* [60]. Short-term (2 days) exposure to $PM_{2.5}$ increases HMGB1 release in the airways of mice [61], which may subsequently lead to airway epithelial barrier disruption. The PM-mediated release of HMGB1 and subsequent ERK-induced barrier disruption may be dependent on ROS levels, as oxidative stress was reported to enhance the release of HMGB1 from epithelial cells *in vitro* [62]. Therefore, ROS may not only lead to EGFR activation, but may also induce the release of HMGB1 and thus increase ERK signalling, leading to disassembly of AJs and TJs (figure 1c).

The oxidative damage induced by air pollutants is thought to be mainly mediated through the mitochondria that are responsible for oxidative phosphorylation (OXPHOS) and concomitant ROS production [63]. Mitochondria are also highly sensitive to oxidative stress damage, and mitochondrial damage leads to higher ROS production. It has been proposed that PAH present in PM triggers oxidative damage to mitochondria [64, 65]. Mitochondrial dysfunction has been reported in the airway epithelium upon environmental exposures [63]. Notably, transcriptomic analysis of AECs exposed to $PM_{2.5}$ revealed a significant alteration in the expression of genes regulating metabolic functions [66]. A metabolic shift from OXPHOS to glycolysis has been observed in AECs stimulated with organic soluble fraction of $PM_{2.5}$ for 24 h [67]. In line with these findings, repeated exposure of AECs from both patients with COPD and non-COPD subjects to $PM_{2.5}$ for 24 h induced mitochondrial dysfunction with increased mitochondrial (mt) ROS levels and reduced OXPHOS activity [68]. Similar mitochondrial abnormalities with enhanced ROS production have been observed in the lung tissues of mice and rats that received $PM_{2.5}$ intranasally for 1 month [69, 70] and $PM_{10–2.5}$ for 3 weeks [71]. Increased levels of mtROS may contribute to airway epithelial barrier disruption through the activation of EGFR and ERK (figure 1c) [9, 70].

Air pollutant-induced mucociliary dysfunction in airway epithelium

Mucociliary clearance is mediated by the airway surface liquid (ASL) as well as ciliary function [72]. Both mucus and hydration of the ASL affect the airway epithelial layer, and both are impacted by cigarette smoke (CS) in patients with COPD [73], and possibly by air pollutants. Ciliary function is dependent on ciliary length, beat frequency and numbers of cilia, all of which are affected in COPD and asthma [74] as well as in response to air pollutants [75]. Diesel exhaust-derived PM has been shown to induce ciliary dysfunction by reducing ciliary beat in AECs from both healthy individuals and patients with asthma [76, 77].

Furthermore, while 24 h exposure with higher concentrations of $PM_{2.5}$ (6 and 12 μg·mm^{-2}) reduced ciliary beat in ALI-cultured nasal epithelial cells, 12 h exposure with lower concentrations of $PM_{2.5}$ (1.5 μg·mm^{-2}) promoted ciliary beating, suggesting involvement of an adaptive response in ciliary beat upon exposure to lower concentrations and shorter PM exposures [78]. Furthermore, genome-wide analysis of human AECs exposed to high doses of $PM_{2.5}$ organic extract showed attenuation of cilia and enrichment of mucus marker genes, suggesting $PM_{2.5}$-induced impairment of mucociliary clearance [79]. In line with this, loss of cilia has been observed in mice exposed to $PM_{2.5}$ for 28 days [80]. Chronic exposure of rats to airborne PM for 7 months induced COPD-like phenotypes in the airways with an increase in mucin 5AC (MUC5AC) expression and mucus metaplasia [81]. MUC5AC expression is partly regulated by the EGFR pathway. Of note, low-dose $PM_{2.5}$ was shown to increase MUC5AC expression in mice trachea and human AECs which was associated with an increase in the EGFR ligand amphiregulin [82]. This PM-induced increase in MUC5AC was further shown to be mediated by activation of the EGFR-PI3K-AKT axis *in vitro* [83]. Additionally, PM from wood smoke increase MUC5AC expression in AECs *via* activation of EGFR and downstream signalling p38/mitogen-activated protein kinase (MAPK), glycogen synthase kinase 3β (GSK3β) and β-catenin [84]. Activation of MAPK and ERK was also associated with PM-induced increase in the expression of MUC5B and MUC5AC in AEC [85, 86]. Oxidative stress may contribute to this mucus hypersecretion in AECs, as ROS-mediated activation of ERK1/2 aggravates MUC5A overexpression in AECs exposed to PM [87]. Nevertheless, mucus hypersecretion could be transiently beneficial in acute exposures, since it efficiently traps PM and impedes its transportation to the epithelium as evidenced by attenuation in ROS in differentiated AECs exposed to iron-rich PM from an underground railway [88]. In line, it was shown that MUC5AC expression was unaffected by acute exposure of AECs to PM; instead, the accumulation of mucus-containing vesicles within the cytoplasm of goblet cells was associated with PM exposure [89]. Therefore, chronic exposure to air pollutants may more pathologically contribute to airway obstruction by inducing ciliary destruction and a shift to hypersecretory phenotype in the epithelium.

Dysregulation of non-coding RNAs: a potential mechanism for air pollutant-induced airway epithelial barrier disruption

Non-coding RNAs, including microRNAs (miRs) and long non-coding RNAs (lncRNAs), are single-stranded RNA sequences regulating many homeostatic processes in the lung such as cellular differentiation, remodelling, host defence and mucociliary function by post-transcriptional modifications of mRNAs [90, 91]. Differentially-regulated miRs have been observed in airway diseases and linked to the pathogenesis of asthma and COPD [92]. More importantly, several miRs, such as miR34c, miR145, miR146a, miR155, miR223 and miR4516, were reported to regulate epithelial barrier function in intestinal and airway epithelium [93–98]. For instance, TGFβ-mediated increase in expression of miR-145, which is also highly expressed in the airway epithelium of patients with asthma and patients with COPD [92, 99], was shown to reduce E-cadherin, β-catenin and claudin-1 gene expression in airway epithelium in mice [98]. Ambient PM is also shown to disturb miRs and lncRNAs regulation in AECs, leading to dysregulated expression of miR-29–3 bp, miR-375 and metastasis-associated lung adenocarcinoma transcript 1 (MALTA1) [100–102]. Extracellular vesicles (EVs) are a group of membrane-derived cellular cargo transporters promoting paracrine signalling during homeostasis and diseases by regulating apoptosis and innate immune responses to foreign particles and pathogens including by transferring miRs [103, 104]. It was shown that ambient PM increases the release of thiol-dependent EVs in AECs *in vitro* [105]. This may alter EV properties, for example, by modifying miR profiles, as it has been observed in CS-stimulated AECs [106, 107]. In addition to miRs, specific lncRNAs have been demonstrated to affect airway epithelial barrier dysfunction, including MALTA1. Notably, ambient $PM_{2.5}$ was shown to reduce E-cadherin gene expression in AEC *in vitro*, in which an NF-κB-mediated increase in MALTA1 expression was proposed to be involved [100, 108]. Moreover, $PM_{2.5}$ may induce airway epithelial barrier disruption *via* another lncRNA, maternally expressed gene 3 (MEG3). Higher expression of MEG3 has been observed in the lungs of patients with COPD and this was associated to disease severity [109]. Both $PM_{2.5}$ and CS induce apoptosis and autophagy in AECs *via* a similar mechanism through MEG3 [109, 110], which may lead to loss of airway barrier integrity. In addition, MEG3 overexpression has been shown to induce barrier disruption in AECs by decreasing E-cadherin gene expression, leading to inhibition of basal cell differentiation to club, goblet and ciliated cells [111]. In summary, these observations show that non-coding RNAs may mediate various of the effects of air pollution on airway epithelial barrier function.

Mechanisms underlying barrier disruptive effects of airborne nanoparticles

The extensive use of nanotechnology has caused a massive increase in engineered nanoparticle production, which has led to increased atmospheric levels of these particles [112]. Evidence suggests that a broad range of physicochemical properties of engineered nanoparticles can influence lung epithelial cell

responses and barrier integrity [113]. Long-term inhalation of atmospheric nanoparticles, such as carbon nanotubes, copper oxide (CuO), titanium dioxide (TiO_2) and silver nanoparticles, potentially causes airway injuries which my lead to the development of asthma and COPD [114]. Of note, CuO nanoparticles that have diverse applications in industrial products were shown to increase mucus secretion *via* MAPK-mediated increase in MUC5AC expression both in healthy donor-derived AECs and in a murine asthmatic model, suggesting a potential contribution of CuO to the pathogenesis of asthma [115, 116]. TiO_2 nanoparticles are the most abundantly produced nanomaterial in commercial products [117, 118]. These particles have been shown to induce dose-dependent inflammation and injury of the lower airways [96, 119]. A recent study showed that TiO_2 nanoparticles also disrupt airway epithelial barrier structure and function through oxidative stress-mediated loss of TJs and AJs as well as inducing inflammation and enhanced multiple pro-inflammatory cytokines *in vitro* and *in vivo* [120]. Furthermore, TiO_2 induces mucociliary dysfunction in AECs through mucus hypersecretion *via* MAPK-mediated increase in expression of MUC5B [121]. *In vitro* studies have shown that SiO_2 nanoparticles disrupt TJs through ROS-mediated activation of ERK [122] and impair ciliary beat activity through inhibition of a calcium-permeable channel in AECs [123]. Aerosolised graphene oxide, an emerging nanomaterial, also impairs airway epithelial barrier function as 1-month exposed ALI-cultured AECs showed reduced TEER levels, which were likely to be mediated through blockage of autophagy and lysosomal-mediated alteration in trans-cellular ions flux (figure 1c) [124]. These studies put forth the notion that nanoparticles not only contribute to the development of airway diseases but may also aggravate pre-existing airway diseases by provoking airway barrier disruption.

It should be noted that many *in vitro* studies used high deposited PM doses on a surface by exposing submerged cultures to concentrated PM [31, 34, 35, 125]. These studies have mimicked PM exposure by using concentrations that reflect exposure accumulated over multiple years in a short exposure model. Therefore, the impact of lower concentrations of PM in a longer exposure time should be considered in future experimental studies.

Immune-mediated alterations in airway epithelial barrier function upon exposure to air pollutants

Air pollutant-induced airway barrier dysfunction may lead to altered immune responses and increased susceptibility to infection, and thus plays a major role in the pathogenesis of asthma and COPD. Immune mediators including cytokines, chemokine and proteases produced by innate immune cells, including AECs, may contribute to airway epithelial barrier disruption. As such, air pollutants may not only directly induce disruption of cell–cell contacts, but may also lead to barrier disruption by stimulating release of these immune mediators (table 1). In particular, long-term exposures to these factors may induce exaggerated pro-inflammatory responses, which may compromise cell contacts. Notably, chronic exposure of rats to PM from biomass fuels induced an inflammatory phenotype with increased neutrophils in the bronchoalveolar lavage (BAL), which was accompanied with airway epithelial barrier disruption [81]. Accumulation of neutrophils in the airway submucosa can lead to epithelial barrier disruption by the

TABLE 1 Immune mediators and host defence molecules involved in air pollutant-induced physicochemical airway epithelial barrier disruption in asthma and COPD

Innate/adaptive immune response	Cytokine/ chemokine	Levels upon air pollutant exposure	Levels in the airways of patients with COPD/asthma	Effects on airway epithelial barrier	Mechanism of effect on barrier	References
Th1	TNF-α IFN-γ	↑ AECs (PM)	↑ COPD	Disruptive	EGFR-mediated ERK activation	[81, 126]
Th17	IL-17A	↑ AECs (PM)	↑ COPD	Disruptive	PM-induced increase in mtROS Increased mucus production	[127–130]
IL-1 family	IL-1β	↑ AECs (PM)	↑ COPD	Disruptive	ADAM17-mediated activation of HER2 Increased mucus production	[127, 128, 131–133]
	IL-33	↑ AECs (ozone, PM)	↑ COPD/ asthma	Protective	Reduction in recruitment of neutrophils	[39, 134]
HDPs	β-defensin 1	↓ AECs (diesel exhaust, PM)	↓ COPD/allergic asthma	Protective	Reduction in bacterial clearance	[135, 136]
	CC16	↓ AECs (PM) ↑serum (ozone)	↓ COPD	Protective	Reduction in bacterial and viral clearance	[137–139]

Th: T-helper cells; TNF: tumour necrosis factor; IFN: interferon; AEC: airway epithelial cell; PM: particulate matter; EGFR: epidermal growth factor receptor; ERK: extracellular signal-regulated kinase; IL: interleukin; mtROS: mitochondrial reactive oxygen species; ADAM17: A disintegrin and metalloprotease 17; HER2: human epidermal growth factor receptor 2; HDP: host defence peptide; CC16: club cell secretory protein 16.

release of neutrophil-derived cytokine oncostatin M and serine proteases, which were shown to disrupt cell–cell contacts in the AECs [140, 141]. $PM_{2.5}$ induces an increase in interleukin (IL)-1β levels through mtROS-mediated NLRP3 inflammasome activation in lung tissue and BAL of mice, which may also trigger airway barrier disruption [142]. IL-1 family members, including IL-1β and IL-33, directly or indirectly regulate airway barrier function [9] and are upregulated in AECs in response to air pollutants [39, 134, 143]. IL-1β induces ADAM17-mediated activation of human epidermal growth factor receptor 2 (HER2) through increased release of neuregulin (NRG1), leading to airway barrier dysfunction as demonstrated by a reduction in TEER and a decrease in claudin18 *in vitro* [131, 132]. Activation of HER2 has also been shown to induce disassembly of AJs by phosphorylating β-catenin leading to segregation from E-cadherin in AECs [133]. The inflammasome is known to subsequently cleave IL-1β into its active form. Inhibition of the NLRP3 inflammasome, NRG1 and HER2 kinase was shown to prevent IL-1β-induced airway epithelial permeability [144]. Higher inflammasome activity has been observed in patients with asthma and patients with COPD [145, 146], and the air pollutants ozone, $PM_{2.5}$ and $PM_{10–2.5}$ have been shown to induce inflammasome activation in the airways *in vitro* and *in vivo* [71, 147, 148].

Moreover, IL-1β and IL-17A augment mucus production by activation of NF-κB subunit p65, leading to hypersecretion of MUC5B and MUC5AC [127, 128], which together with air pollutant-induced ciliary dysfunction may aggravate airway obstruction. By contrast, several cytokines were shown to induce epithelial barrier protective effects and yet their release was increased upon exposure to air pollutants. IL-33 is such a cytokine that was shown to be released upon exposure to ozone, diesel exhaust and $PM_{10–2.5}$ [39, 149] and to exert airway epithelial barrier protective effects in ozone-induced lung injury model *in vivo* by restoring expression of E-cadherin, ZO-1 and claudin-4 proteins possibly *via* regulating neutrophil recruitment [39].

Activation of adaptive immune system may contribute to airway epithelial barrier dysfunction. T-helper cytokines that play a key role in pathogenesis of asthma and COPD, such as those produced by T-helper (Th)1, Th2 and Th17 cells, have been shown to contribute to epithelial damage [9, 150]. Exposure to air pollutants has been shown to activate these adaptive immune responses and increase release of their mediators, including pro-inflammatory cytokines such as IFN-γ, TNF-α, IL-13, IL-17A and IL-22 (figure 1b) [81, 129, 149, 151]. The receptors of these cytokines are expressed by AECs and all of these cytokines have been reported to induce airway epithelial barrier disruption upon CS exposure [9]. For instance, the expression of Th1-type cytokines IFN-γ and TNF-α was induced by air pollutants in airways of rats [81], and both cytokines have been shown to disrupt TJs in AECs *in vitro* through EGFR-mediated activation of ERK [126]. Air pollutant-induced Th17 cytokines IL-17A and IL-17F may also disrupt airway barrier function [9]. PM exposure induced an upregulation in IL-17A in AECs *in vitro* [129]. IL-17A upregulation was shown to suppress E-cadherin expression in AEC *in vitro* [130]. $PM_{2.5}$-induced elevation of IL-17A in AECs has also been shown to promote mitochondrial dysfunction, which may lead to increased mtROS levels and further reduction in cell integrity [130]. Furthermore, IL-22 as a key effector in Th17 differentiation was also shown to contribute to airway barrier dysfunction by reducing E-cadherin in AECs from patients with severe asthma [152]. IL-22 levels increased in the airways of both patients with severe asthma and severe COPD [152, 153]. The barrier disruptive action of IL-22 only occurs with TGF-β co-stimulation [152], suggesting the additive action of IL-22 on TGF-β, as exogenous IL-22 increases TGF-β expression in the lungs of mice [154]. Interestingly, IL-22-gene deleted mice had improved barrier function with decreased IFN-γ in BAL [155], further suggesting that complex interaction with other cytokines may choose the effect on barrier function. Mice exposed to urban PM for 4 days also showed a AhR-dependent increase in IL-22 in their lungs, which may affect barrier function [156]. Th2-driven responses as a dominant phenotype in allergic asthma have also been linked to dysregulated airway barrier function [150]. This Th2 phenotype is also observed upon exposure to air pollutants as observed by an increase in Th2 cytokines IL-4 and IL-13 upon PM exposure [149, 151]. Notably, mice that chronically inhaled $PM_{2.5}$ showed disrupted sinonasal epithelial barriers as demonstrated by a decrease in E-cadherin and claudin-1 expression, which was accompanied with an increase in IL-13 in the sinonasal epithelium [143]. IL-13 and IL-4 were shown to disrupt airway epithelial barriers *in vitro* through the Janus-associated kinase [157]. Together, dysregulated pro-inflammatory and immune responses upon long-term exposure to air pollutants may exacerbate airway epithelial barrier dysfunction in patients with asthma and COPD.

Air pollutant-induced airway epithelial dysfunction and susceptibility to microbial infection

Dysregulated innate immune responses and susceptibility to respiratory infections

Disrupted airway epithelium with impaired innate immune responses as a consequence of exposure to air pollutants may increase the risk of airway infections by facilitating respiratory pathogens to invade the

epithelium [9, 158]. ROS-mediated epithelial barrier disruption induced by air pollutants may directly enhance pathogen entrance through a more permeable epithelium. Indeed, it was shown that PM exposure increases internalisation and colonisation of *Pseudomonas aeruginosa* in airway epithelium, which was associated with ROS-mediated disassembly of TJs [159]. In addition to increasing bacterial internalisation through disruption of barrier function, air pollution impairs airway epithelial antimicrobial responses to respiratory pathogens leading to decreased clearance of pathogens and disease exacerbation (table 1). Airway epithelial-derived host defence peptides (HDPs) are a group of antimicrobial agents released into the extracellular space in response to pathogens [30]. Altered secretion of certain HDPs such as β-defensins has been observed in patients with asthma and patients with COPD as well as in response to noxious particles and was associated with pathogenesis of the diseases [135, 160–162]. Whole diesel exhaust was shown to downregulate host defence peptide β-defensin-2 in AECs from patients with COPD stimulated with *Haemophilus influenzae* [136]. In line with these observations, PM was shown to decrease airway epithelial HDPs and in particular β-defensin1/2 and stimulate *P. aeruginosa* growth *in vitro* and *in vivo* [163], which was reported to be mediated by ROS [158]. More clinically relevant, a study revealed that children exposed to $PM_{2.5}$ in a polluted area had decreased airway epithelial-derived salivary agglutinin, an antimicrobial glycoprotein, suggesting that PM-exposed individuals may potentially be more vulnerable to respiratory infections. PM also reduces *P. aeruginosa* clearance in the airway epithelium by altering HDPs and impairing mucociliary function *via* thyroid transcription factor 1 which regulates club cell-derived HDPs [164]. In line, PM was shown to enhance respiratory syncytial virus (RSV) infection in mice by attenuating club cell-derived secretory proteins (CCs) [137]. This air pollutant-induced impairment in HDPs may lead to increased inflammatory responses to pathogens as observed in the airway epithelium of patients with asthma exposed to PM and RSV [165]. Additionally, PM upregulates host cell surface proteins that can be hijacked by respiratory pathogens to colonise and enter the epithelial cells. Indeed, it was shown that $PM_{10–2.5}$ increases attachment of *Streptococcus pneumoniae* to the airway epithelium *via* an increase in platelet-activating factor receptor *in vitro*, thereby promoting airway infection [166]. Furthermore, nitrogen dioxide as a gas component of ambient air pollution increases airway epithelial expression of intercellular adhesion molecule-1 (ICAM1) [167], which is exploited by major pathogens involved in COPD exacerbation, *i.e.* rhinovirus and non-typeable *H. influenzae*, to attach to AECs [168, 169]. Interestingly, high expression of ICAM1 was notable in the goblet cells of patients with airway complications [170], suggesting a potential link between air pollutant-induced increase in pathogen attachment and mucus overproduction in COPD exacerbation. The air pollutant-induced susceptibility to respiratory pathogens upon exposure to air pollutants may either be caused by or lead to altered composition of microbiota in the airways.

Air pollutant-induced dysbiosis in lung microbiome

Over the past decade, the involvement of the lung microbiome in the maintenance of respiratory health and the development and progression of respiratory disease has gained an increasing amount of attention. The core constituents of the healthy human lung microbiome are the phyla Bacteroidetes and Firmicutes, with *Prevotella* (Bacteroidetes), *Streptococcus* and *Veillonella* (Firmicutes) as the most abundant genera (figure 2a) [171]. The lung microbiome composition can be influenced by environmental and lifestyle factors. In contrast to the gut, where decreased microbial richness has been associated with disease, in the lung increased diversity has been associated with disease, including asthma [172]. Moreover, alterations in the composition of the bacterial constituents in the lung has been observed in different lung disease states including, but not limited to, asthma, COPD, cystic fibrosis, respiratory infections and pulmonary fibrosis [173]. Of note, overrepresentation of Proteobacteria and Firmicutes has been reported in the lung of both asthma and COPD patients [9, 174]. Understanding the influence of airborne toxicants on lung microbiome composition is an area of ongoing research. However, to date there are only few descriptive studies which provide the first insights in this field. Exposure of experimental animals to high doses of $PM_{2.5}$ *via* intratracheal instillation was shown to increase diversity and richness of the lung microbiome when compared with medium dose-treated controls [175, 176]. Specifically, in rats, Proteobacteria were decreased following $PM_{2.5}$ exposure, whereas Bacteroidetes, Cyanobacteria and Firmicutes increased significantly with increasing $PM_{2.5}$ concentrations [176]. Normal lung microbiome in mice and rats predominantly include Proteobacteria following with other phyla such as Actinobacteria, Firmicutes and Bacteroidetes in rat and Bacteroidetes, Cyanobacteria and Firmicutes in mice [175, 176]. In contrast, chronic exposure of mice to lower doses of concentrated $PM_{2.5}$ was shown to decrease richness and diversity of the lung microbiome compared with that in mice who were exposed to filtered-air [177]. In humans, short-term exposure to ozone led to a decreased airway epithelial barrier function as observed by an elevation in serum CC16 and an altered composition of nasal flora with a decrease in abundance of Firmicutes and Actinobacteria [138]. In line, short-term exposure to both $PM_{2.5}$ and $PM_{10–2.5}$ was negatively associated with abundance of Firmicutes, Actinobacteria and Proteobacteria in human nasal passage [178]. Moreover, children living in polluted areas had higher microbial diversity in the sputum than those living in less-polluted areas (figure 2b) [179]. The $PM_{2.5}$-induced changes in the sputum

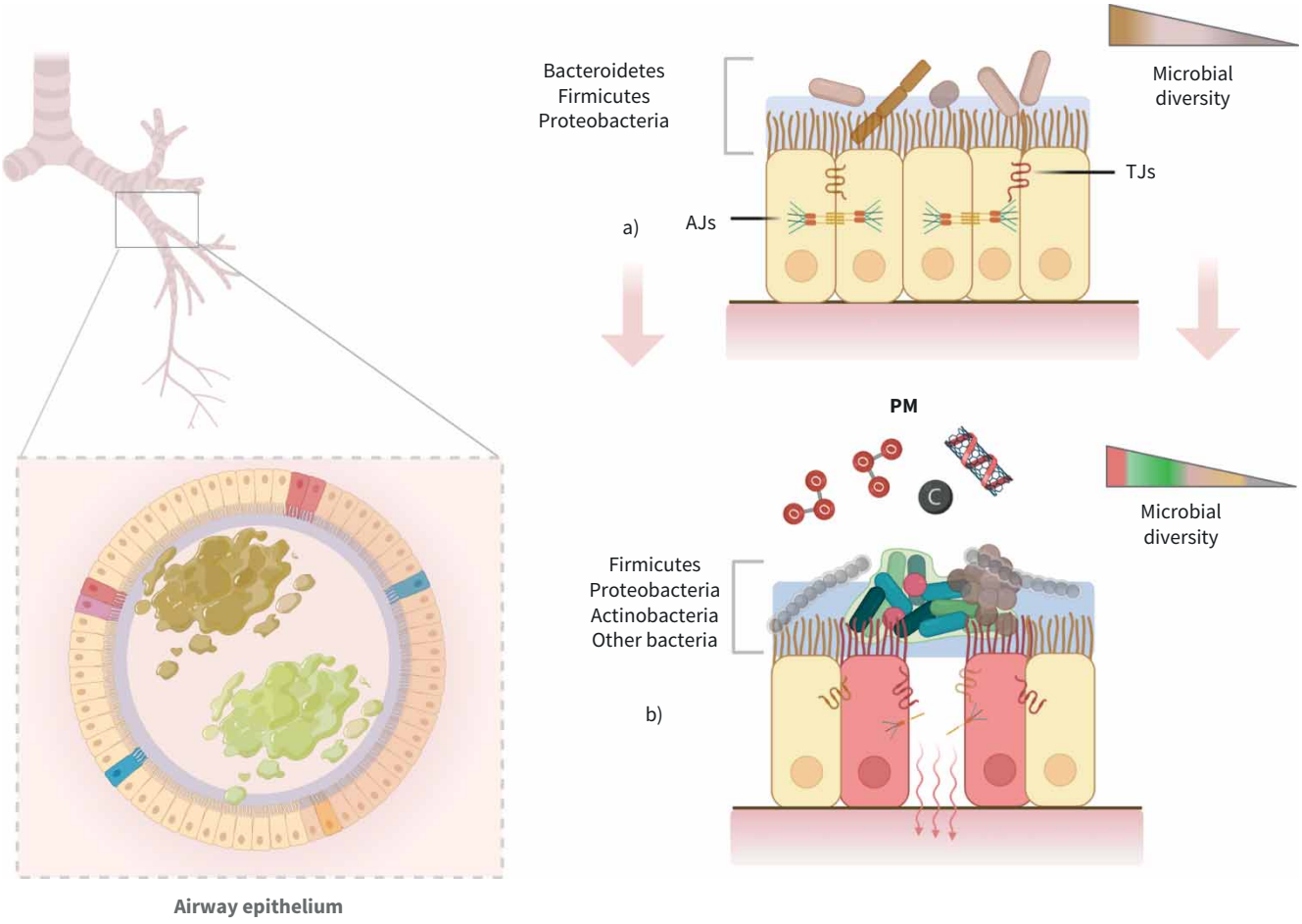

FIGURE 2 Air pollutant-induced microbial dysbiosis in airway epithelium may enhance airway epithelial permeability. a) Healthy human airway microbiome is mainly composed of Firmicutes, Proteobacteria and Bacteroidetes. b) Upon acute exposure to various air pollutants, the composition of the microbiome in the upper airways changes, with an increased abundance of Firmicutes, Bacteroidetes and Cyanobacteria in rats, while fewer Actinobacteria, Proteobacteria and Firmicutes are observed in humans. PM induces dissemination of microbiome from upper to the lower airways where it exerts pathogenic actions, as observed by the abundance of Firmicutes and Proteobacteria in the lower airways of individuals exposed to PM. PM-induced dysbiosis in lung microbiome may induce airway barrier disruption as pathogenic Firmicutes and Proteobacteria, including *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*, that are known to disrupt airway barriers are overrepresented in the lower airways, which may exacerbate pre-existing epithelial damage in patients with asthma and patients with COPD. AJ: adherens junction; PM: particulate matter; TJ: tight junction. Figure partially created with BioRender.com.

microbiome has been linked to a decline in respiratory function, and these changes lasted for at least 14 days following exposure [180]. In addition, $PM_{2.5}$ has been suggested to increase the risk of developing respiratory infections [181], and thus may lead to alterations in the lung microbiome and promote acute exacerbations of asthma and COPD, which are often associated with microbial infections. Intriguingly, there is evidence suggesting that $PM_{2.5}$ can carry both airborne bacteria and bacteria-derived components to the lung [182]. However, whether this directly contributes to changes in the lung microbiome and the development of respiratory infections or exacerbations of chronic lung diseases warrants further research. A shift in microbiota towards Proteobacteria and Firmicutes including *S. pneumoniae*, *H. influenzae* and *P. aeruginosa* has been observed in the sputum of patients undergoing COPD exacerbations. Several of these pathogens have been reported to induce an increase in the permeability of AECs [9]. In addition to outdoor pollutants such as PM, household air pollution in Malawi was linked to alterations in the lung microbiome, with elevated proportions of *Neisseria* and *Streptococcus* in the lung [183], although bacterial diversity and relative abundance at the phyla level were not significantly different. Overall, the air pollutant-induced airway dysbiosis may provide a therapeutic insight into controlling airway epithelial barrier dysfunction in asthma and COPD.

Therapeutic strategies for restoration of airway epithelial barrier function in air pollutant-induced asthma and COPD

Due to the complex pathogenesis of airway diseases involving a plethora of factors, single therapy may not be as effective as combinational therapy. Although combinational therapy with inhalational corticosteroid (ICS) and long-acting bronchodilators (LABA/LAMA) is routinely prescribed to asthmatics and patients with COPD to control airway obstruction and inflammation, its potential to reverse barrier disruption induced by air pollutants is not evident. Budesonide, as a FDA-approved corticosteroid frequently used in asthma and COPD to suppress inflammation, failed to show any effect on $PM_{2.5}$-induced barrier dysfunction in ALI-cultured AECs [35]. Moreover, tiotropium, a widely used LAMA, was unable to reduce excessive ROS produced in response to PM in an ovalbumin-induced mouse model of asthma [184], suggesting that it may not be effective on ROS-mediated barrier dysfunction. Therefore, considering supplementary therapeutics aimed at restoring airway epithelial barrier function may counteract mucosal and submucosal damage induced by air pollutants and as such may improve lung function in patients with progressive airway diseases. This barrier protective strategy can be triggered by inhibiting excessive oxidative burden, reducing autophagy and suppressing pathways involved in hyper-inflammatory response. Of note, eliminating PM-induced excessive ROS in nasal epithelial cells of mice by ROS scavengers including N-acetyl-cysteine (NAC) has been described to restore TJ assembly *in vivo* [32]. In line with this, pre-treatment of nasal epithelial cells with NAC exerted similar protective effects on TJs in response to $PM_{2.5}$ *in vitro* [159, 185]. Interestingly, treatment with an antioxidant was effective in inhibition of AhR activation as the upstream regulator of ROS production in AECs in response to air pollutants [48], which may thus also restore defects in epithelial barrier function. Furthermore, it may suppress pro-inflammatory signals leading to barrier dysfunction, as excess ROS activates NF-κB signalling by phosphorylating the IκB subunit and increasing translocation to the nucleus, which may trigger further barrier dysfunction by inducing pro-inflammatory cytokine release [186]. Treatment with NAC also reduced PM-induced increase in ROS in AECs *in vitro*, which subsequently inhibited IκB phosphorylation and release of pro-inflammatory cytokines [186]. Furthermore, oral pre-treatment of mice with the macrolide Azithromycin was shown to reduce airway barrier dysfunction induced by short-term inhalation of SO_2 by suppressing inflammatory responses [38]. Inhibition of autophagy and ROS production by an antioxidant resveratrol may be an additional effective strategy to simultaneously overcome PM-induced cell junction disruption and mucus overproduction in AECs [125]. Resveratrol as an antioxidant and sirtuin 1 activator restored airway epithelial integrity *via* targeting mitochondrial function and reducing ROS, thus it may be similarly effective against air pollutant-induced airway epithelial damage [187, 188]. Furthermore, co-inhalation of hydrogen gas as an antioxidant, which has already been shown to improve lung function in a CS-induced rat model of COPD [189], was shown to reduce MUC5AC expression and oxidative damage in the airways of rats exposed to $PM_{2.5}$ *via* amelioration of AhR-Nrf2 [190]. Lower levels of dietary antioxidants such as α-tocopherol have been observed in the airways of individuals exposed to air pollution [191], while their intake has been shown to suppress the air pollution-induced decline in lung function in patients with asthma [191, 192]. This suggests that receiving sufficient antioxidants in the diet may help to prevent respiratory disease in patients living in polluted areas, although controlled dietary intervention studies are needed to further support this conclusion. In addition to antioxidants, vitamin D3 (VitD3) supplementation may also restore airway epithelial barrier function by upregulating the expression of TJ proteins and in a broader sense by stimulating epithelial HDPs production [193]. Low levels of VitD3 observed in the serum of infants and women have been associated with high levels of air pollution [194, 195], making it a potential target for therapy. Of note, 1 day pre-treatment with VitD3 diminished $PM_{2.5}$-induced increase in ROS and activation of NF-κB in AECs [196], which may lead to reduction in ROS-mediated barrier damage. Furthermore, VitD3 supplementation suppressed TGFβ-induced decrease in E-cadherin in AECs and inhibited epithelial-mesenchymal transition (EMT) [197]. In addition, transcriptomic analysis of AECs exposed to PM revealed that VitD3 reduces PM-induced increase in claudin7 expression as a TJ component that known to promote EMT in intestinal epithelial cells [198, 199], suggesting a protective role for VitD3 against EMT. Together, employing these therapeutic strategies along with conventional ICS and LABA/LAMA may reduce the mucosal damage and improve lung function in the patients with asthma and COPD, particularly for those who reside in highly polluted areas.

Conclusion

Inhalation of PM and noxious gases that are emitted into the air in cities on a daily basis is a risk factor for the development of asthma and COPD. Current knowledge on the pathogenesis of these lung diseases confirms the negative impacts of air pollutants on lung function in patients with airway diseases. Airway epithelial barrier disruption is one of the central features of asthma and COPD, and air pollution is considered to be a major trigger for its development. We have summarised the key mechanisms regulating airway epithelial barrier disruption upon exposure to various air pollutants, of which ROS-mediated mechanisms appear to be the common mechanism. Airway epithelial barrier dysfunction induced by air

pollutants perpetuates inflammation and airway remodelling and increases susceptibility to infections which may explain the higher rate of exacerbations observed in the patients with asthma and patients with COPD living in polluted areas. As high levels of urban gas phase air pollutants were shown to be particularly associated with occurrence of COPD exacerbations [200], it is essential to widely scrutinise the impacts of these pollutants on airway epithelial barriers. Although changes in the lung microbiome induced by air pollutants may facilitate airway infection and as such exacerbations, the direct link with barrier dysfunction is unknown and requires further investigations. Furthermore, due to the role of viral and fungal pathogens in airway epithelial barrier dysfunction, it is relevant to investigate the impact of air pollutants on the lung virome and mycobiome and to delineate how putative changes may contribute to barrier dysfunction. Restoring barrier function by therapeutic compounds, particularly those suppressing excessive ROS production by AECs, such as resveratrol and for instance VitD3 in combination with the routine ICS/LABA/LAMA medications, may be an effective strategy to prevent development of new cases as well as exacerbations in current patients with asthma and patients with COPD residing in polluted areas.

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