Airway Epithelium Plays a Leading Role in the Complex Framework Underlying Respiratory Allergy

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Abstract

Airway epithelium is the cellular structure with the greatest surface exposed to a plethora of environmental airborne substances, including microorganisms, respiratory viruses, air pollutants, and allergens. In addition to being a protective physical barrier at the air–liquid interface, the airway epithelium acts as an effective chemical and immunological barrier that plays a crucial role in orchestrating the immune response in the lungs, by supporting the activation, recruitment, and mobilization of immune cells. Airway epithelium dysfunction has been clearly associated with various airway inflammatory diseases, such as allergic asthma. Although it is not fully understood why a person develops respiratory allergy, a growing body of evidence shows that the nature of the host’s immune response is strongly determined by the state of the airway epithelium at the time of contact with the inhaled allergen. Our review highlights the physiological state of airway epithelium as a key element in the development of allergy and, particularly, in exacerbation of asthma. We review the role of physiological oxidants as signaling molecules in lung biology and allergic diseases and examine how high exposure to air pollutants (eg, cigarette smoke and diesel particles) can contribute to the increased incidence of respiratory allergy and exacerbation of the disease.

Key words: Airway epithelium. Barrier dysfunction. Respiratory allergy. Redox biology. Air pollutants.

Resumen

El epitelio pulmonar constituye la barrera celular más susceptible a la acción deletérea de la multitud de agentes que se encuentran en el ambiente, incluidos los alérgenos. Además de prevenir su acceso al organismo, la barrera epitelial de las vías respiratorias juega un papel inmunomodulador crucial, regulando de forma local la acción de las células del sistema inmune subyacentes. Una disfunción epitelial, provocada tanto directa como indirectamente por la acción de los aeroalérgenos, parece ser una de las causas principales de desregulación de la homeostasis pulmonar, causando una respuesta proinflamatoria descontrolada que cada vez más autores atribuyen al origen de las reacciones alérgicas. En esta revisión se quiere destacar el papel de la barrera epitelial pulmonar como regulador de la respuesta inmune en el contexto de la alergia. Las enfermedades crónicas que afectan a las vías respiratorias, tales como el asma alérgica, muestran frecuentemente una función epitelial defectuosa, apoyando así la hipótesis antes mencionada que subyace al origen de la alergia. El impacto de otros contaminantes ambientales -como virus respiratorios, bacterias, humo del tabaco y partículas diésel- sobre la integridad epitelial, así como su influencia en la biología redox pulmonar relacionada con el desarrollo de la respuesta alérgica, también se abordarán en la presente revisión.

Introduction

The lung is a complex internal organ that is widely exposed to airborne substances (e.g., microorganisms, respiratory viruses, air pollutants, and allergens), which are frequently expelled without inducing lung inflammatory responses because of the crucial role of the airway epithelium in the regulation of immune homeostasis and the development of defense mechanisms.

Over the last few decades, increasing evidence has indicated an association between a dysfunctional epithelium and airway inflammatory diseases [1], of which respiratory allergy is considered a key inflammatory disorder of the conducting airways. Respiratory allergy is characterized by a dominant CD4+ T-helper 2 (TH2)–type immune response and by the production of high levels of specific IgE against antigens called aeroallergens (Figure 1). Pollen from trees, weeds and grasses, mold spores, house dust mite fecal particles, and animal dander are common sources of aeroallergens. These particles use the upper airways as the main portal of entry into the body, with the airway epithelium being not only the protective physical barrier that they have to overcome, but also an active immunochemical capable of orchestrating allergen-associated immune responses [2]. It is not fully understood why a person develops respiratory allergy, although a growing number of studies suggest that this clinical disorder is the result of a complex interplay between genetics and the environment [3].

Although the debate about whether an impaired airway epithelium can be the cause of allergy—as opposed to the consequence—remains unresolved, a growing body of evidence indicates that the nature of the host immune response is strongly determined by the developmental state of the epithelium at the time of contact with the inhaled allergen [4].

Moreover, several studies have addressed the role of oxidative stress in allergic inflammation (Figure 2) [5]. The lung epithelium is continuously exposed to oxidants generated during normal metabolism or present in the environment (e.g., ozone, nitrogen dioxide, car exhaust emissions, and cigarette smoke). Besides the deleterious effects of these molecules, physiologic oxidants have been shown to be signaling molecules and key regulators of allergic responses in the lung [6,7].

Our review focuses on the airway epithelium as the key orchestrator in the complex framework underlying respiratory allergy. We examine the role of the airway epithelium as a key...
player in orchestrating the allergen immune response, the role of lung “redox biology” in respiratory allergic inflammation, and the effect of air pollutants on the airway epithelium and respiratory allergies.

**Airway Epithelium as a Key Player in Orchestrating the Allergic Immune Response**

Besides its role as a selective and highly regulated physical barrier through the establishment of the intercellular apical junctional complexes between neighbouring airway epithelial cells (AECs) [8-11], airway epithelium also acts as a critical orchestrator of the airway immune response through the secretion of a wide repertoire of molecules, including antimicrobial peptides and proteins (eg, lysozyme, defensins, and protease inhibitors), antioxidants (eg, superoxide dismutase and glutathione), chemokines, and cytokines, in response to environmental stimuli [2,12-14].

AECs have been identified as the main source of interleukin 25 (IL-25), IL-33, and thymic stromal lymphopoietin (TSLP), the so-called triad of AEC-derived cytokines. These molecules operate upstream of the canonical TH2 cytokines, primarily IL-4, IL-5, and IL-13, which play a pivotal role in allergy (Figure 1) [15]. At the mucosal surface, the triad of AEC-derived cytokines are important regulators of maintenance of immune homeostasis and induction of both a protective and an inflammatory TH2-type immune response [16-18]. Dysregulation of these cytokines can lead to exacerbated recruitment and activation of specific immune cells, such as dendritic cells (DCs), type-2 innate lymphoid cells (ILC2s), mast cells (MCs), eosinophils, neutrophils, and basophils, and thus promote a long-term TH2-type airway hyper-responsiveness spiral. Increased levels of these cytokines have been widely detected in asthma patients [19].

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**Figure 2. Overview of the molecular mechanisms involved in allergen-triggered immune responses in the context of the lung epithelial redox homeostasis.**

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced endogenously by eosinophils, neutrophils, and airway macrophages, resulting in S-glutathionylation of reactive cysteine residues within a protein, thus further controlling its activity. Air pollutant interaction with the airway epithelial barrier constitutes an alternate source of oxidants that finally lead to a tissue redox imbalance, enhancing allergic symptoms as stated above. Endogenous and exogenous oxidant species are constantly controlling redox biology in the lung; their levels may act in favor of or against the development of allergic disease. GSH indicates reduced glutathione; Grx, glutaredoxin; GSTpl, glutathione-S-transferase pi; H2O2, hydrogen peroxide; NO, nitric oxide; O2, superoxide; ONOO-, peroxynitrite; P-SH, protein thiol; P-SOH, protein sulfonic acid; P-SSG, protein S-glutathionylation; SOD, superoxide dismutase; Srx, sulfiredoxin; Trx, thioredoxin.
Expression and secretion of IL-33 (IL1F11) and IL-25 (IL-17E) are highly regulated in AECs [20–22]. Both cytokines act as amplifiers of the allergic inflammatory immune response [23,24] via the membrane receptors IL-33R and IL-17RA/IL-17RB, respectively, which are highly expressed in various immune cells, including ILC2s, MCs, basophils, DCs and macrophages [25–29]. In particular, IL-33 can stimulate MC degranulation [30] and release of IL-8 [31], IL-1β, IL-6, tumor necrosis factor-alpha (TNF-α), and chemokine (C-C motif) ligand (CCL) 2 [32], which lead to formation of leukotrienes and prostaglandins and can enhance the inflammatory environment in the lungs [33]. In addition, IL-33 also induces expression of IL-9 in freshly isolated basophils and CD4+ T cells [34]. IL-25 is able to induce promotion of the Tn2 lineage via activation of DCs [35] and is involved in the proliferation of a subpopulation of inflammatory ILC2s [36]. Finally, IL-33 and IL-25 also increase basophil activation and migration in allergic asthma patients [37], and high levels of both cytokines have been reported in bronchoalveolar lavage fluid in asthma models [38].

TSLP is highly secreted by AECs in response to allergens, viruses, diesel extract particles, microbes, and helminths via Toll-like receptor (TLR) signalling [39]. Its secretion is also promoted by loss of E-cadherin after injury and exposure to TNF-α. IL-13, IL-6, IL-9, IL-17, and amphiregulin [45], which was recently shown to perpetuate allergic inflammation [46]. TSLP can also modulate several functions of MCs [47], including the release of proinflammatory cytokines such as IL-6, TNF-α, and IL-1β, but not cell degranulation [41]. Finally, TSLP has been associated with susceptibility to multiple allergic diseases by regulating basophil hematopoiesis [48].

AECs also release granulocyte-macrophage colony-stimulating factor (GM-CSF), CCL20 and CCL2 [49,50], IL-1α, and IL-4 or IL-13 [51] in response to house dust mite allergens (mite extract and Der p 1). These cytokines can induce chemotaxis of MCs and DCs in the epithelium, activation, and migration of DCs to lymph nodes, and polarization of Tn2 cells [52]. Human and mice bronchial epithelial cells can also produce IL-1α in response to allergen exposure and have an autocrine effect that potentiates the secretion of the aforementioned cytokines [53]. Eotaxins 1, 2, and 3 delivered by AECs are also able to induce activation of eosinophils and Tn2 in asthma [54]. In addition, allergen-exposed AECs secrete other alarms, such as adenosine triphosphate, uric acid and amphoterin (HM-GB1) [55], which are generally released in stress and cell death responses. Increased levels of these molecules have also been reported in patients with asthma [56]. In conclusion, the secretory role of the airway epithelium in orchestrating innate and adaptive immune responses suggests that impaired epithelial barrier function may trigger uncontrolled and deleterious inflammatory processes, such as those observed in allergic reactions.

Aberrant immunochemical actions of the airway epithelium are ultimately determined by the maintenance of the structural integrity of the barrier. Thus, the barrier may be potentially altered by a primary cell-cell junction disruption or alternatively as a consequence of an epithelial remodeling process or epithelial-mesenchymal transition process, both of which involve cell-cell contact disassembly and an increase in the expression of mesenchymal-associated proteins such as α-smooth muscle actin, vimentin, and/or fibronectin [57-59]. The immediate effect of epithelial-mesenchymal transition on barrier function is easier access by inhaled allergens across a more permeable epithelial barrier, thus enabling their interaction with local immune cells. A repetitive injury resulting from chronic exposure to allergens may lead to persistent activation of airway repair processes or, in the worst case scenario, altered migration of progenitor cells and abnormal epithelial dedifferentiation, thus further contributing to unbalance the polarized secretion of the proinflammatory epithelium-derived molecules. Airway remodeling-derived inflammation in response to allergens has been widely associated with chronic respiratory diseases such as asthma [60,61] and has been shown to be even more persistent by means of a prolonged allergen challenge in mice [62-64]. Sustained allergen exposure also promotes the persistence of mucous cell hyperplasia in the murine airway wall [65], as well as smooth muscle remodeling in an experimental model of asthma [66].

Various features of a dysfunctional innate immune function of AECs appear to persist in cultures of cells from asthmatic patients, indicating underlying genetic and epigenetic mechanisms [67]. Polymorphisms in IL-6 [68], IL-4 [69], high-affinity IgE receptor [70], IL-4R [71], disintegrin and metalloproteinase 33 [72], and IL-13 [73] have been associated with epithelial dismantling and malfunctioning, especially in the context of asthma and chronic obstructive pulmonary disease [74,75]. In this sense, a high level of IL-13 in asthmatic patients has been involved in wall remodeling processes [76,77]. Moreover, some genetic variants of TSLP have been linked to asthma or allergic rhinitis [78]. The altered genes that appeared in exposed individuals have been implicated in pulmonary effects driven by environmental agents such as viruses, cigarette smoke, oxidants, and air pollution [79-81], explaining, in part, the higher susceptibility of these patients to the action of airborne insults (see below).
a highly reactive RNS form of peroxynitrite (ONOO⁻) [82]. All these molecules play a relevant role in many cellular processes, such as proliferation, survival, airway remodelling, mucus secretion, and apoptosis and are also critical in the development of airway inflammatory processes [83].

Since all aerobic living forms suffer oxidative damage, the lungs present several antioxidant systems to maintain redox homeostasis. Under normal physiologic conditions, the balance between generation and elimination of ROS/RNS maintains the functional integrity of cell structures and the redox state of signalling proteins. However, prolonged exposure to oxidants can cause dysregulation of redox homeostasis, leading to “oxidative stress”, which can in turn lead to cell death and has been proposed as the origin of a variety of airway diseases, such as allergic asthma (Figure 2) [84].

Endogenous oxidants are generated not only by mitochondrial respiration, but also by inflammation-activated cells such as eosinophils, neutrophils, monocytes, macrophages, and tissue-resident cells including endothelial, alveolar, and bronchial epithelial cells. As for the role of mitochondria in inflammation, oxidative stress may cause release to the cytosol of compounds such as damage-associated molecular pattern molecules, adenosine triphosphate, cardiolipins, and mitochondrial DNA, subsequently activating immune signalling cascades via intracellular activation of TLR agonists [85]. Mitochondrial dynamics are directly altered by the presence of airborne particles and protease allergens, leading to changes in membrane potential and proteosomal activity that ultimately leads to proapoptotic events [86,87]. However, the most important source of endogenous oxidants comes from the activation of inflammatory, immune, and structural cells by environmental components (including inhaled allergens), which in turn regulate the expression of specific genes for proinflammatory mediators and antioxidant protective enzymes via activation of redox-sensitive transcription factors such as activator protein-1 (AP-1) and NF-kB. In addition, inflammatory cells present a variety of oxidant-generating enzymatic systems that amplify the inflammatory response and oxidative stress initiated by exogenous insults, thus contributing to lung damage. Neutrophils, macrophages, and eosinophils produce O₂⁻, NO, NO₂⁻, and H₂O₂ by NADPH oxidase (gp91phox-NOX2), eosinophil peroxidase (EPO), myeloperoxidase (MPO), and inducible NO synthase (iNOS) [88]. In AECs, NADPH oxidase dual oxidase 1 (DUOX1) catalyzes the generation of ROS and regulates IL-33 secretion and the subsequent T₃,2-immune response to allergen challenge [89].

Besides having a direct deleterious effect on most of the cellular components, leading to lipid peroxidation of membrane phospholipid, depletion of intracellular tripeptide glutathione (γ-glutamyl-cysteinyl-glycine [GSH]), DNA damage, and amino acid modification in cellular proteins [90], ROS/RNS also have a critical role in the activation of redox/signalling pathways and transcription factors (see above). Modifications to proteins by physiological oxidants mainly involve reversible oxidation of cysteine residues, which act as cell redox sensors that regulate homeostasis and various biological processes, among them cell proliferation, migration, differentiation, and signalling, as well as gene transcription [5]. In contrast, protein modifications by exogenous oxidants are often associated with the pathogenesis of many human diseases, including asthma, heart disease, neurodegenerative disease, and cancer.

GSH, which contains a sulfhydryl group, is a critical antioxidant in the lung, in particular in the protection of airway epithelium against damage induced by oxidants and inflammation. GSH is the predominant antioxidant in airway cells, ranging from 1 to 11 mM depending on the subcellular compartment, and its concentration in the epithelial lining fluid (ELF) that bathes the entire surface of the lung, is over 100-fold higher than in plasma [91-93]. GSH is a key player in the maintenance of redox homeostasis in the lung, which is mainly defined by the ratio of the concentration of its reduced form to its disulfide form, GSH/GSSG [94]. Under normal conditions, the oxidized form represents less than 1% of the total GSH pool; however, alterations of the GSH/GSSG ratio in ELF have been described in patients with asthma and other inflammatory disorders such as cystic fibrosis. In this sense, depletion of GSH from the ELF activates inflammatory routes involving NFκB activation, thus playing a key role in the expression of proinflammatory genes and cytokine release [95]. In animal models, exposure to toxins, viruses, or allergens such as OVA decreased the total thiol content in bronchoalveolar lavage fluid, thus inducing epithelial apoptosis, cytokine release, and eosinophil influx. However, a higher GSSG content was observed, together with impaired activity of various enzymatic antioxidants such as superoxide dismutase (SOD), catalase, and thioredoxin [96,97]. Lower antioxidant activities, including those associated with GSH synthesis, have also been described in ELF and in cells of asthmatic patients. On the other hand, nuclear factor (erythroid-derived 2)–like 2 is a transcription factor that regulates the expression of phase II and antioxidant enzyme genes, for example, catalase, SOD, glutathione-S-transferase (GST), glutathione peroxidase (GPx), and glutathione synthetase, and is considered a feedback loop that is originally affected by dramatic changes in the GSH/GSSG ratio [97]. In this sense, GSTP1- and SOD-specific loci have been strongly associated with asthma and deficiencies in oxidant defenses, although their role remains unclear [98]. As for the protective role of GSH, it is noteworthy that GSH can also be reversibly incorporated into cysteine residues of proteins, through an enzymatic process called S-glutathionylation [99]. This process, which is catalyzed mainly by GSTs, protects oxidant-target proteins from being irreversibly oxidized, thereby avoiding their aberrant activation or loss of functionality through proteasome degradation [100]. This post-translational modification can alter the activity and function of specific transcription factors involved in inflammatory processes, such as the p65 and p50 subunits of NFκB, thus inhibiting their binding to DNA [101,102] and avoiding the expression of numerous proinflammatory genes. The regulatory inhibitory kappaB kinase beta may also undergo S-glutathionylation, thus allowing the inhibition of NFκB transactivation [103]. The expression of other inflammatory genes, such as c-Jun (AP-1), has also been reported to undergo S-glutathionylation [104]. Finally, the rate of S-glutathionylation is markedly lower in patients with asthma [105], thus reinforcing the hypothesis that associates the redox imbalance with the development of
the pathological features of severe allergic asthma, including airway inflammation and epithelial barrier impairment.

Effect of Air Pollutants on the Airway Epithelium and Respiratory Allergies

Its anatomical and functional features make the lung the organ with the largest surface exposed to a wide range of air pollutants from natural sources or man-made activities, which have a harmful impact on human health and also on animals and plants. Air pollutants include volatile organic compounds, particulate matter (PM), gases, and metals [106], with carbon monoxide (CO), hydrocarbons, nitrogen dioxide (NO₂), PM, (SO₂), and ozone (O₃) being the most common and abundant. Whilst O₃ is formed in the earth's atmosphere by UV light reactions, CO, NO₂, and PM are generated from fossil fuel burning from motor vehicles, factories, gas heaters, cookers, and cigarette smoke, all of which are concentrated in urban areas. For example, in major cities, up to 90% of airborne PM consists of diesel exhaust particles, which are high-molecular-weight carbon-based entities (~100 nm) that can promote allergic airway inflammation and hyper-responsiveness [107-109].

Air pollutants can facilitate sensitization to aeroallergens by a direct effect on airway epithelium and largely contribute to respiratory allergic symptoms and to the pathogenesis of other chronic airway diseases, in particular chronic obstructive pulmonary disease [110,111]. Air pollutants act by increasing airway epithelial barrier permeability, inhibiting mucociliary clearance, and inducing AECs to secrete an array of inflammatory mediators such as chemokines, cytokines, eicosanoids, and adhesion molecules that recruit and activate DCs, ILC2s, and basophils, thus contributing to T-h2-type polarization [116]. Furthermore, numerous studies have shown that smokers exhibit impaired mucociliary clearance [117-120] because of the inhibitory effect of cigarette smoke compounds on the expression of genes involved in ciliogenesis [121]. In turn, an increase in barrier permeability may facilitate the access of aeroallergens to the submucosa and their interaction with resident DCs and ILC2s, as well as with recruited MCs, eosinophils, lymphocytes, and neutrophils [122].

The deleterious effect of air pollutants on airway epithelium has been attributed to oxidative stress [123]. Air pollutants can also generate ROS, which activates NF-κB signalling in the airway epithelium, leading to the secretion of the epithelial cytokines IL-1, IL-25, IL-33, GM-CSF, and TSLP, as well as other mediators. Subsequently a variety of cellular events are promoted and result in allergic sensitization to inhaled allergens [124,125]. O₃ [126], NO [127], and cigarette smoke [128,129] induce epithelial cells to release ROS and RNS.

Another mechanism by which air pollutants contribute to respiratory allergies is by interacting with allergens, thus acting as carriers and adjuvants that modify the features of allergens, which in turn enhance the immune response to them. It has been shown that PM and diesel exhaust particles can carry allergens from pollen, cat, dog, and house dust mite [130]. Moreover, it has been reported that air pollution increases the allergenic potency of pollen [131-133]. In their model based on guinea pigs sensitized to Zinnia pollen, Chelegani et al [134] observed that pollen collected from polluted urban areas not only induced higher levels of serum IgE, but also increased eosinophil counts compared with nonpolluted pollen. Finally, growing evidence indicates that air pollutants can increase the production of allergens and proinflammatory mediators in pollens as a result of the plant adaptive response to environmental stress [131,135]. In addition, it has been shown that pollens are a source of oxidants [136]. Once pollen NADPH oxidases reach the airway epithelium, levels of ROS and GSSG in ELF increase. These NADPH oxidases, which originally participate in pollen germination and pollen tube formation, could contribute to the allergic airway inflammation induced by the allergen.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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