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STATE-OF-THE-ART REVIEW

The Impact of Early-Life Exposure to Air-borne Environmental Insults on the Function of the Airway Epithelium in Asthma

Kirsten Spann, PhD, Natale Snape, PhD, Engin Baturcam, BSc, MSc, Emmanuelle Fantino, PhD
Queensland, Australia

Abstract

The airway epithelium is both a physical barrier protecting the airways from environmental insults and a significant component of the innate immune response. There is growing evidence that exposure of the airway epithelium to environmental insults in early life may lead to permanent changes in structure and function that underlie the development of asthma. Here we review the current published evidence concerning the link between asthma and epithelial damage within the airways and identify gaps in knowledge for future studies.

KEY WORDS asthma, allergens, air pollution, virus, epithelium, early life, airway remodeling, immunity

INTRODUCTION

Asthma has a complex etiology and has been viewed conventionally as being caused by a dysregulated adaptive immune response[1] for which individuals are genetically predisposed. However, in more recent years the contribution of both the airway epithelium and the innate immune response to the development of asthma in early life has been considered. The airway epithelium is the interface between the internal environment of the lungs and the inhaled environment. It not only provides a physiological barrier critical in directly protecting the airways from environmental insults, but also is an intrinsic part of the innate immune response to respiratory challenges. Airway epithelial cells (AECs) are capable of producing a wide range of cytokines and chemokines that activate immune cells. The response of the airway epithelium to environmental insults has a lasting effect on respiratory health and plays a critical role in the early-life establishment of chronic respiratory disease. Recurrent or severe exposure to environmental insults during the first years of life may induce lifelong changes to the structure and function of the airway epithelium. Moreover, a growing body of evidence links epithelial damage with the development of asthma in early life.[2,3]

The *exposome* is a term recently used to encompass all of the environmental insults humans are exposed to during the first years of life.[2] For the respiratory system, these include pathogens, allergens, and pollutants. However, chronic respiratory illnesses are not only the result of early-life exposures. Genetic factors also influence the effect of the exposome on the development of respiratory disease.[3]

In this review, we consider the evidence that the early life exposome has an effect on the structure

From the School of Biomedical Sciences, Queensland University of Technology, Queensland, Australia (KS); and Children's Health Research Centre, University of Queensland, Queensland, Australia (NS, EB, EF). Address correspondence to K.S. (kirsten.spann@qut.edu.au).

and long-term function of the airway epithelium. We discuss the main mechanisms by which these environmental insults induce long-term dysregulation of the structure and function of the airway epithelium. We focus on in vitro research that has been carried out using cultured primary AECs of bronchial or nasal origin. However, studies using transformed cell lines, primarily BEAS-2B and 16HBE14o cells, will be discussed. Immortalized human lung alveolar cells, A549s, have traditionally been the predominant cell line used, however, they of limited relevance being derived from a carcinoma. Most studies using primary AECs have used submerged monolayer cultures, which are composed of epithelial cells in basal morphology. In recent years, air-liquid interface (ALI) cultures have been used as they mimic a physiological airway and therefore allow the investigation of barrier function and repair. Mouse and monkey models have been included in this review where they have been used directly to investigate epithelial cell responses.

THE AIRWAY EPITHELIUM IN EARLY LIFE

The airway epithelium is the first line of defense against environmental insults. When functioning normally, it forms a physical barrier of stratified ciliated epithelial cells, mucus-secreting goblet cells, and surfactant-secreting clara cells. The formation of tight junctions at the apical surface of the columnar cells and other adhesion mechanisms along the basal surface ensure an impermeable barrier. Tight junctions are formed by interacting adhesion proteins such as ZO 1-3, occludin, claudins 1-5, E-cadherin, and β-catenin[4,5] in addition to extracellular matrix (ECM) interactions.[6] However, this essential barrier between the external environment and the lung is not fully formed at birth. Infant lung alveolarization is not complete until 2 to 4 years of age. During this time, the airway epithelium is vulnerable to environmental challenges that can alter genetic and epigenetic determinants of lung function, induce airway remodeling, and reduce the long-term capacity of the airways to repair.[7]

In addition to perinatal physiological lung development, the immune system is also developing in the neonate.[7] The lack of a functional adaptive immune response makes infants highly susceptible to infections and dependent on the innate immune response. As such, the residing immune cells of the airway epithelium and epithelial cells themselves are a vital part of the innate immune response to environmental insults of the airways and long-term reprogramming of these cells may mediate lifelong chronic disease.[1]

VIRUSES

There is a long-standing debate concerning the link between viral infections in early life and the inception of asthma. However, growing experimental and clinical evidence shows that early-life exposure to severe and repeated viral infections causes episodic airway inflammation, which leads to a cycle of tissue damage, repair, and remodeling. Over time, this may lead to persistent pathological changes in the epithelium.[7,8] The principal viruses that cause early life wheeze are human rhinoviruses (HRVs), respiratory syncytial virus (RSV), and human metapneumovirus (hMPV).[9] For pediatric cohorts, research concerning the effect of viral infection on the airway epithelium has been focused mainly on HRV and RSV, whereas the long-term effect of early-life infections by hMPV is largely unknown.

Several cohort studies[10-13] consisting of infants hospitalized for respiratory infections during their first year of life have identified a significantly increased risk for asthma at 5 years of age. In particular, RSV bronchiolitis in early life has been linked to the development of wheeze and asthma, with cohort studies demonstrating that up to 50% of children who experience severe RSV bronchiolitis in infancy develop asthma.[13-17] The strongest evidence that RSV-induced wheeze and bronchiolitis predispose infants to the later development of asthma comes from clinical trials of anti-RSV treatments. A nonrandomized trial in which late-preterm infants were prophylactively administered anti-RSV antibodies demonstrated a 68% to 80% reduction in the risk for recurrent wheeze, thus demonstrating a causative link between RSV and early-life wheeze.[18] Furthermore, a randomized control trial with 429 preterm infants found that prophylactively administered anti-RSV antibodies reduced the duration of wheezing by 61% during the first year of life.[19] The causative mechanisms that underlie the link between RSV and asthma are not entirely clear. It is believed that early-life RSV infections may elevate susceptibility to repeat viral infections via the dysregulation of immunologic pathways, long-term epithelial damage, and airway remodeling. A strong association also exists between HRV infections and lower respiratory illness in children, with infants hospitalized for HRV-induced wheeze having increased

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risk for the development of asthma.[10,12,20] However, as with RSV, the role of HRV in airway remodeling during the first years of life is not clear.

Although RSV and HRV have been specifically identified as being linked to early-life wheeze and later development of asthma, a recent prospective birth cohort where frequency of respiratory episodes was adjusted, revealed that the development of asthma during childhood was associated with the number of respiratory viral infection in early life, regardless of the viral trigger.[21] Using the same cohort, it was demonstrated that duration of wheezy episodes was independent of the type of virus, but rather due to the interactions of the virus and host factors such as immunologic susceptibility.[22] The precise role particular viruses have in the induction of long-term changes in epithelial function is therefore unclear.

ALLERGENS

Allergens react with specific immunoglobulin E antibodies to induce an allergic, or atopic, state. Although genetic factors may predispose to atopy, the development of atopic asthma is dependent on environmental exposure and sensitization to allergens in early life.[23] Indoor allergens are predominantly house dust mite (HDM), pet hair, molds, and cockroaches. Fungi are both indoor and outdoor allergens, whereas pollen is an outdoor allergen. Early exposure to HDM has been associated with the development of asthma, where one particular study identified that exposure to $\geq$10 μg/g dust mite allergen/total dust was associated with increased risk for asthma and wheeze at age 7.[24] In addition to early exposure, continual exposure to HDM has been shown to contribute to the development of airway hyper-responsiveness and asthma in early childhood.[25] The effect of cat and dog hair on allergic sensitization is not clear, with some studies finding a correlation between early-life exposure and subsequent wheezing,[26,27] whereas others identified a protective effect for developing asthma.[28,29] Cockroach allergens have been strongly associated with asthma onset. In several studies worldwide, early exposure to cockroach allergen has been associated with a higher incidence of wheeze and early-onset asthma.[30-32] Mold and fungi have been associated with increased risk for asthma in children.[33,34] The most frequent respiratory allergens are pollens, particularly from grasses, followed by certain species of tree, such as birch and oak. Grass pollen allergens have been linked to asthma exacerbations as well as allergic rhinitis and hay fever. Allergic sensitization and the functional consequences of prolonged allergen exposure in the airways increase with damage to the epithelium caused by other factors. Furthermore, allergens themselves can have a deleterious effect on the barrier function of the airway epithelium.

OUTDOOR AIR POLLUTION

The health effects of indoor and outdoor air pollution have been studied widely, with a growing global population exposed to dangerous levels. Children are particularly susceptible to the effects of air pollution, not only due to underdeveloped lung physiology and immune responses, but also due to behavioral factors. Higher incidence of mouth breathing, longer times outside engaged in physical activity, and the breathing of air nearer the ground where toxicants settle all lead to exposure of children to elevated doses of pollutants.[35] Air pollution has been linked to increased severity of childhood respiratory infections and the development of childhood asthma.[36-38] The main source of outdoor air pollution is fuel combustion from vehicles and other industrial activities, including carbon monoxide (CO), nitrogen dioxide (NO_2), sulphur dioxide (SO_2), and polycyclic hydrocarbons (PAH). SO_2 is released by industrial activities where fossil fuels are either burned or processed, and also by diesel engines. In the airways, where SO_2 is exposed to water, it forms sulphurous (H_2SO_3) and sulphuric (H_2SO_4) acid, both of which can induce bronchoconstriction and bronchospasm in asthmatics.

Another potent pollutant is ozone (O_3), which is formed when NO_2 reacts with organic compounds.[39] Ozone is released by burning fossil fuels and in highly urbanized areas by the action of sunlight on volatile organic compounds and nitrogen oxides. Ozone is a powerful oxidizing agent and is used commercially as a disinfectant. In a recent study, infant rhesus monkeys were exposed to acute and episodic O_3 during the first 2 months of life.[40] This resulted in airways that were hypersensitive to O_3 at 6 months of age, as well as elevated inflammation and cell death in the distal airways.

In addition to these gasses, air pollution contains particular matter (PM), which is a mix of solid and liquid particles suspended in the air. Living in close proximity to PM sources is linked to reduced lung function and increased allergen sensitization.[41,42] Diesel exhaust particles (DEPs) are a key source of inhaled PM and have been long linked to asthma. Several epidemiologic studies have shown increased

prevalence of asthma in people who live closer to major roads.[43] In particular DEPs can trigger T-helper (Th)2 immune responses. Furthermore, some gases, such as SO_2, can adhere to other pollutants to form PM.

INDOOR AIR POLLUTION

The adverse health effects of passive exposure to cigarette smoke early in life have been extensively studied. Approximately 38% of children are exposed to environmental tobacco smoke (ETS) in the home.[44] This exposure to "side-stream" smoke, primarily from parental smoking, doubles the risk for respiratory infection.[45,46] In one study, parental smoking was attributed to 200 to 500 excess hospitalizations and 1000 to 5000 diagnoses of respiratory infections per 100,000 children.[47] Cigarette smoke consists of many chemical constituents including gases and PM. These include various hydrocarbons, nicotine, CO, nitric oxide, hydrogen cyanide and various pro-oxidative heavy metals.[48] Exposure of the airways to this array of toxicants in early life leads to epithelial dysfunction and is a major risk factor for the development of asthma.

Indoor air pollutants other than ETS include combustible biomass for heating, lighting, and cooking; phthalates from dust and carbon dioxide; volatile organic compounds; and other chemicals used in cleaning products.[49] The effect of these pollutants on the airway epithelium in early life is not well studied compared with the effect of outdoor pollutants and ETS. In fact, the link between indoor pollutants and the development of asthma is not conclusive. Most studies concerned with indoor air pollution have used mouse models and very few have investigated the response of the airway epithelium. However, there are some limited studies using in vitro epithelial cell cultures.

MECHANISMS OF AIRWAY INJURY

Asthma is traditionally considered a chronic disease due to skewed Th2 polarization of the immune inflammatory responses. However, disruption and remodeling of the epithelial mucosal barrier has an equally significant pathogenic role in the etiology of asthma.[50] Environmental insults cause epithelial cell fragility and shedding, loss of apical polarity of epithelial cells, goblet cell metaplasia, and the deposition of ECM proteins, such as collagen, which result in subepithelial fibrosis, smooth muscle hypertrophy and hyperplasia, and increased angiogenesis.[51] This airway remodeling results in the narrowing of airways and airway wall thickening and underlies airway hyper-responsiveness in chronic respiratory diseases such as asthma. Airway remodeling appears to occur within the first years of life as several studies of preschool children have demonstrated that the characteristics of airway remodeling can be observed before a clinical diagnosis of asthma.[52] There is growing and compelling evidence that airway remodeling and chronic airway epithelial injury during the first years of life, while the lungs are still developing, underlies the onset of asthma in childhood. The mechanisms by which environmental insults play a role in airway remodeling and dysregulated epithelial function in early life are discussed further.

Environmental insults do not act in isolation, but rather interact to cause long-term damage to the epithelium via a range of processes. Additionally, several mechanisms of airway injury may occur as a result of one or more simultaneous environmental insults. However, most in vitro studies using primary AECs investigate isolated mechanisms of epithelial damage to single insults. We consider these mechanisms individually, although we also discuss studies in which the interactions of environmental insults have been considered.

THE LOSS OF EPITHELIAL BARRIER FUNCTION

The loss of epithelial barrier function exposes the basal lamina to damaging particles such as air pollutants, tobacco smoke particles, allergens, and pathogens and allows these insults to penetrate the airways. This induces a range of tissue damage responses including inflammation and cell death. If subsequent tissue regeneration is ineffective, remodeling occurs within the airways that may result in long-term epithelial barrier disruption. Consequently, this would allow access for inhaled triggers of airway hyper-responsiveness.

One of the most useful models for the investigation of epithelial barrier function is the establishment of well-differentiated ALI cultures of primary AECs. In this model, apical cells are ciliated and regenerate from a basal cell layer, and goblet cells produce mucus to replicate the human airways. Using this model, environmental factors have been shown to disrupt the tight junctions between epithelial cells that are essential for barrier function. HRVs induce loss of barrier function of the airway epithelium through loss of junctional

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plaque protein and zona occludens-1.[53] In another study, RSV infection led to a significant reduction in transepithelial electrical resistance (TEER) of the tight junctions and remodeling of the apical actin cytoskeleton.[54] As RSV and HRVs are primary causes of wheeze in infants and young children, such epithelial damage has long-term implications for respiratory health. Allergens produce proteinases that can have a direct effect on the barrier function of the airway epithelium. For example, HDM proteinases activates PAR-2, which is a proinflammatory receptor that leads to E-cadherin destabilization and loss of tight junction integrity.[55,56] Pollen grains[57,58] and fungi[59] also produce proteinases that induce tight junction degradation. Cockroach allergen effects epithelial permeability via the release of vascular endothelial growth factor (VEGF) from epithelial cells.[60]

The loss of epithelial barrier function as a result of ETS exposure has been studied using cigarette smoke extracts (CSE). Similarly to allergens and viruses, CSE has been reported to increase epithelial cell permeability by reducing the structural integrity of tight junctions.[61-63] This can occur via downregulation of genes that encode junction proteins, and also by dislocating junction-associated proteins from the cell membrane.[63] Exposure to CSE further inhibits the function of the mucociliary escalator, which lines the airway surface and entraps pathogens for expulsion. CSE reduces ciliary beating, denudes the epithelium of cilia, and causes mucus hypersecretion and squamous cell metaplasia.[64] All of these factors reduce the ability of the epithelium to expel pathogens.

The inhalation of O_3 results in the death of ciliated and type I epithelial cells. These death processes in asthmatics may be skewed toward necrotic inflammatory cell death. Rhesus monkeys have been used to investigate the effect of O_3 exposure on the airway epithelium. In one study, exposure of 2-month-old rhesus monkeys resulted in injury and necrosis of epithelial cells, the release of inflammatory mediators, and the influx of neutrophils and eosinophils in the distal airways.[40] The columnar epithelium in these monkeys was obviously disrupted and displayed irregular morphology. Early-life exposure to O_3 also changed the pattern of programmed cell death such that these airways were hyperresponsive to O_3 challenge at 6 months of age.

One of the key mechanisms in epithelial repair is the infiltration of macrophages into the airways. This initiates inflammatory processes that remove injured cells, and assist in the clearance of cellular debris and ECM repair.[65] In a recent study, asthmatic and nonasthmatic volunteers were exposed to inhaled O_3 and the subjected to bronchalveolar lavage (BAL) to investigate gene expression in BAL cells. Most genes and proteins upregulated in the BAL of the individuals with asthma were involved in immune and inflammatory responses and cytokine activity, wounding, adhesion, and matrix remodeling. In individuals with asthma, the secreted protein upregulated the most was osteopontin (OPN), which is reported to play a role in tissue repair and remodeling in animal models.[66] In this same study a semi-immortalized bronchial epithelial cell line, 16HBE14o, was used in a scratch test model of wound repair to show that the ratio of polymeric and monomeric forms of OPN is critical to wound repair, with the polymeric form playing a greater role in repair. This same study demonstrated that individuals with asthma exposed to increasing doses of O_3 produced less polymeric and more monomeric OPN, indicating a defect that may explain reduced epithelial wound repair in asthma after exposure to O_3.

AIRWAY REMODELING

Excessive expression of epithelial growth factors is linked to deposition of fibrotic material within the airway wall. This is a key feature of early-life airway remodeling, which leads to long-term airway thickening and hyper-responsiveness (AHR).[67] AHR is defined as heightened responsiveness to specific or nonspecific stimuli. Spasmogens, such as histamine, activate the airway smooth muscle (ASM) to shorten, causing narrowing of the airway lumen and increased resistance to airflow; typical of asthma. The ASM surrounding the airways is typically enlarged in asthmatic airways. This may be caused by many factors including intrinsic defects, although dysregulated expression of mediators by environmental insults has been implicated in the enlargement of ASM mass. ASM cells themselves can release proliferative and proremodeling factors, which can act in an autocrine fashion to promote enlargement of ASM mass.[68-71] However, damaged epithelial cells release cytokines and metabolic factors that also act on ASM.

One of the principal mechanisms of airway remodeling and increased ASM mass is a process called epithelial-mesenchymal transition (EMT), which is central to the role of the epithelium in asthmatic inflammation and remodeling in early life.[50] The factors that drive EMT are complex and directly affected by environmental exposures.

In the case of epithelial injury due to exposure to environmental irritants, growth factor secretion by epithelial cells is elevated in an attempt to transform specialized epithelial cells into fibroblast-like cells to promote repair. These resident growth factors include epithelial growth factor (EGF), fibroblast growth factor (FGF), and transforming growth factor (TGF)-β. However, rather than promoting epithelial repair, excessive growth factor expression results in the loss of apical polarity, reduced cadherin and disruption of epithelial junctions. Transformed epithelial cells may secrete excessive collagen and fibronectin, and promote airway remodeling by increased ASM mass.

RSV is known to induce TGF-β1 and also SNAI1, which is a regulator of EMT.[72] Aero-allergens are strong inducers of EMT. For example, HDM induces myosin light-chain phosphorylation and actin reorganization, enhances β-catenin-Wnt pathway and ultimately leads to TGF-β–induced EMT.[73] Timothy grass pollen has been demonstrated to induce TGF-β, although in A549 cells, not primary epithelial cells.[74]

Early-life exposure to environmental insults can induce excessive expression of growth factors by AECs. In cultured primary AECs, HRV has been shown to induce the production of FGF-2, the EGF amphiregulin, and also the TGF activin A.[75-77] These factors have been linked to subepithelial fibrosis in asthma.[77] Primary AECs infected with HRV also release the angiogenic factor VEGF.[76,78] The balance between matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of MMP-9 (TIMP-1) contributes to regulating airway remodeling.[79,80] However, MMP-9 is elevated in the airways of individuals with asthma, hence this imbalance may promote airway remodeling. In primary AECs cultured in vitro there is evidence that HRV induces the expression and activation of MMP-9.[79] Moreover, BAL fluids (BALF) from adults with asthma and natural HRV infections were shown to contain elevated VEGF[76] and MMP-9 levels, which correlated to elevated viral load.[79] RSV is also a potent inducer of MMP-9. A recent study of a cohort of children who were mechanically ventilated for RSV disease, found that MMP-9 was elevated in tracheal aspirates compared with MMP-9 levels in a control population. A large proportion of the MMP-9 was in the active form and resulted in an imbalance in the MMP-9-to-TMIP-1 ratio at 48-hour intubation. In this same study, human lung epithelial cells were cultured at ALI and infected with RSV lab strain A2. Furthermore, RSV stimulated MMP-9 release early in infection before loss of epithelial barrier integrity.[81] Elevated MMP-9 release in the BALF has been demonstrated in RSV mouse models.[82]

Air pollutants also induce the expression of airway remodeling factors in epithelial cells, although the use of primary AECs in culture, rather than immortalized cell lines, is limited. Two studies have used the HPV-18 immortalized human bronchial epithelial cell line BEP2D[83,84] to demonstrate that exposure to SO_2 resulted in elevated EGF, EGF receptor (EGFR), and the mucin expression gene *MUC5AC*, which are involved in repair and mucus production. Cyclooxygenase-2 was also elevated in response to SO_2 exposure, and has been associated with increased prostaglandin D_2, thought to cause ASM constriction. Furthermore, intercellular adhesion molecule-1 and interleukin (IL)-13 were elevated and both have been associated with inflammation and hyper-responsiveness in asthma. Another study used the immortalized lung alveolar cell line A549 to investigate the effect of volatile organic compounds present in airborne PM. Results showed that toluene, benzene, and styrene present in the PM induced prostaglandin secretion (PGE[2] and PGF[2α]), which is known to be involved in airway remodeling.[85] DEP has been linked to airway remodeling. One published study challenged primary AECs from healthy and children and children with asthma cultured at ALI with PM suspensions. The baseline concentration of VEGF was significantly higher in cells from the group with asthma compared with healthy children and after PM challenge, asthmatic cells produced more *MUC5A5* and IL-8.[86] The effect of O_3 exposure has been studied using cultured primary bronchial epithelial cells, demonstrating that O_3-induced inflammation was due to EGRF-mediated pathways, rather than nuclear factor-κB pathways, as was traditionally thought

OXIDATIVE STRESS

An important mechanism by which all environmental irritants cause injury to the airways is via the induction of reactive oxygen species (ROS). ROS cause oxidative stress, which leads to the release of proinflammatory cytokines and chemoattractants. The link between early-life oxidative stress in the epithelium and the induction of asthma in vivo has not been directly demonstrated. Nevertheless, functional polymorphisms in oxidative defense genes have been linked to increased risk for developing asthma in childhood,[87,88] and support the

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association. There are several sources of oxidative stress it in the airways.[89] One of these is the infiltration of inflammatory cells after exposure to environmental triggers. These immune cells generate anion superoxide, which is then is dismutated to hydrogen peroxide by superoxide dismutase (SOD) enzymes. The hydrogen peroxide can then generate hydroxyl radicals or hypohalides, in addition to elevated nitric oxide (NO) via upregulation of epithelial inducible NO synthase (iNOS). These reactive molecules affect protein structure and function within epithelial cells that can be long lasting and contribute to chronic disease. Mitochondrial dysfunction within the epithelial cells, as a result of mechanical and environmental stimuli, also results in the formation of anion superoxide.

RSV, hMPV, HRV, and influenza are all known to induce elevated ROS in the airway epithelium. In particular, RSV infection causes ROS and injury to in vitro cultured primary AECs.[90] Air pollution can cause oxidative stress and epithelial damage through direct oxidative injury from gas phase pollutants such as O_3, SO_2, and nitrogen oxides. These pollutants also promote airway inflammation and the infiltration of leukocytes. SO_2, for example, can induce oxidative stress in epithelial cells by producing ROS directly, or by inducing secretion of ROS from infiltrating leukocytes.[91] Epidemiologic studies in children have shown close association between sulphur dioxide emissions and increased thiobarbituric acid-reactive substances, as well as between fine PM, exhaled NO, and sputum IL-8 levels.[92,93] In vitro studies using cultured human bronchial epithelial cells (HBECs) have shown that DEP-induced oxidative stress has a greater effect on AECs than on other cells such as pulmonary macrophages.[94] In a separate study using cultured HBECs, DEP-induced oxidative stress resulted in the release of thymic stromal lymphopoietin (TSLP), which activated selective notch pathways to polarized co-cultured myeloid dendritic cells (DCs) toward a Th2 response.[95]

Pollen has been shown to induce mitochondrial dysfunction in AECs that result in the release of ROS in the airway epithelium. Pollen-induced oxidative stress may activate DCs and lead to differential T-cell phenotypes that predispose to allergic airways diseases.[96,97]

Exposure to cigarette smoke during early life is one of the most significant sources of oxidative stress in the developing airway epithelium. Acrolein is an oxidant produced in cigarette smoke as well as in wood burning and heating of cooking oils. It can be detected in side-stream smoke at levels 17 times greater than in mainstream smoke, which has implications for the exposure of children to smokers. Intranasal exposure of mice to acrolein resulted in elevated activated macrophages in the lungs and elevated ROS formation in these cells. When acrolein-treated macrophages were co-cultured with A549 cells, elevated ROS induced apoptosis of the A549 cells.[98] Adenosine is a factor produced in the lungs that balances tissue repair with excessive airway remodeling. Primary bronchial AECs cultured at ALI, demonstrated a significant delay in wound healing when exposed to CSE followed by physical wounding. It was identified that the CSE impaired adenosine-driven wound healing by inducing oxidative stress via ROS.[99] Another study showed that CSE caused an imbalance in the oxidative response in cultured small AECs obtained by bronchoscopy from donors.[100]

Oxidative stress is controlled by a range of enzymatic and nonenzymatic antioxidant systems that buffer the lung from a wide range of environmental oxidants.[89] An inability to neutralize ROS by cellular antioxidant systems can cause extensive cellular and tissue damage, leading to bronchoconstriction, AHR, enhanced mucous secretion, and epithelial cell damage. Inadequate antioxidant responses are a hallmark of chronic respiratory disease. Environmental insults activate oxidative stress not only by inducing ROS, but also by suppressing the antioxidant response. RSV suppresses antioxidative enzymes (AOEs) via elevated SOD2 expression, thus exacerbating oxidative stress. This has been demonstrated not only in vitro,[90] but also in children with severe RSV-induced bronchiolitis.[101] Furthermore, hMPV has been shown to significantly affect AOE expression in cultured AECs and in a mouse model via elevated SOD2 expression.[101,102] Glutathione (GSH) in the epithelial lining fluid protects the epithelium by reducing hydrogen peroxide levels. When BEAS-2B cells were exposed to O_3 they demonstrated significantly impaired GSH homeostasis required for optimal activity.[103] GSH expression requires binding of the transcription factor Nrf2 to antioxidant response elements. When infected with RSV, mice deficient in Nrf2 had lower levels of active GSH in the lungs, high levels of protein and lipid modification, and suffered more severe inflammation and epithelial injury.[104]

ALTERATION OF THE IMMUNE RESPONSE

Early-life exposure to environmental factors during a time in which the immune system is developing

leads to reprogramming of immune responses via epigenetic modification of gene expression.[8] Damage to the airway epithelium, by oxidative stress or loss of barrier function, allows environmental insults to penetrate this protective barrier and come into contact with immune cells. Whether this induces changes in the immune response during the first years of life that lead to the consequent development of asthma, or if asthmatics are genetically predisposed to an adverse immune response is not clear.

It is well known that infants have a Th2 bias and a limited capacity to produce Th1 cytokines. This early-life Th2 environment has been shown to induce IL-13–driven mucous cell hyperplasia within the airway epithelium and render the epithelium more susceptible to RV infection.[105] This in turn induces further epithelial damage and exposure of environmental insults to immune cells that drive the Th2 inflammatory response. It has been demonstrated in several human nasal challenge studies that DEP can induce sensitization to allergens more effectively than exposure to the allergen alone.[106,107] It is believed that DEP may promote DC maturation during allergic sensitization, and trigger a Th2 response which is directly associated with the development of allergic asthma.[108]

Driven primarily by interferon (IFN) and cell death responses, AECs are inherent immune cells capable of a robust and effective innate immune response, in addition to the secretion of cytokines and chemoattractants that activate further innate immune pathways and inflammation. For example, allergen-epithelium interactions result in the release of cytokines and chemokines that provide a favorable environment for the establishment of a Th2 environment. Allergens stimulate the release of cytokines such as TSLP,[109] IL-33,[110] and IL-25[111] from AECs, which act on DCs to induce *OX40L* expression, which is required for a Th2 phenotype.

Environmental insults also modify the expression and release from epithelial cells of cytokine and chemokines that recruit and activate other immune cells. For example, natural killer (NK) cells play an important role in the airway epithelium by killing pathogen-infected cells and releasing cytokines and chemokines critical for the early immune response. A co-culture model of nasal epithelial cells (NECs) and NK cells was established to identify how O_3 modifies the interactions between these cells types. It was found that O_3-exposed NECs were less able to stimulate IFN-γ release by NKs cells and also reduced their cytotoxicity.[112] In a separate study, AECs were isolated from 1-year-old monkeys that had been exposed to O_3 during the first 6 months of life and then challenged with lipopolysaccharide (LPS) at 1 year.[113] AECs from the O_3-exposed monkeys produced less IL-6 and IL-8 after postnatal O_3 exposure than AECs from monkeys exposed to clear air. Conversely, on challenge with LPS, AECs from the O_3-exposed monkeys produce more IL-6 and IL-8 than AECs from monkeys exposed to clear air. Furthermore, O_3-exposed AECs displayed a different expression pattern of miRNAs capable of binding to and dysregulating IL-6 and IL-8 responses. This indicates that early-life exposure to O_3 can reprogram the inflammatory response of the airway epithelium.

A study using HBECs cultured at ALI investigated the effect of cook stove emissions using a novel aerosol-to-cell deposition system. The emissions from the cleaner cook stoves induced significantly less proinflammatory cytokines than emissions from traditional open fires.[114] This was due to cleaner burning stoves generating less PM and gaseous pollutants than open fires. The effect of PM on epithelial inflammation has been studied in the context of indoor pollution. In one such study, air samples were taken from classrooms in Germany and used to treat BEAS-2B lung epithelial cultures.[115] Indoor PM induced inflammatory genes such as IL-6 and IL-8. However, outdoor PM collected in the same study induced xenobiotic metabolizing enzymes. This study concluded that indoor PM induced more inflammatory and allergic reactions than outdoor PM. The effect of SO_2 in epithelial inflammation has been studied, although in A549 cells, in which several asthma-controlling drugs reduced IL-8 expression after exposure to SO_2.[116] Exposure to SO_2 also increased neutrophil adhesion to A549 cells.[117]

Rather than environmental challenges, such as viruses, inducing changes in the innate immune response of AECs, there is some evidence that intrinsic immune defects underlie susceptibility of the airway epithelium to viral infections. Some research in this area has been conducted using HRV infections of submerged ex vivo cultured primary AECs, although few studies have been conducted using AECs from young children. Early work[118-120] identified that bronchial AECs from asthmatic adults and severe therapy-resistant asthmatic children, were highly susceptible to HRV infection when cultured ex vivo as submerged monolayers. This correlated to significantly reduced

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production of IFN-β and IFN-λ in response to HRV infection. However, not all studies have shown the same response of AEC to virus infection.[121,122] Also, studies in which ex vivo cultures of AECs are exposed to other viruses, such as RSV or hMPV, are limited. A recent study in which submerged monolayers of nasal and tracheal AECs from wheezy and nonwheezy children (ages 2-10 years) were exposed to either RSV or hMPV demonstrated a lack of correlation between shed virus, IFN production, and history of recurrent wheeze.[123] Innate immune defects in asthma are not restricted to the airway epithelium and there is certainly substantive evidence that deficient IFN production and skewed cytokine production by immune cells such as plasmacytoid dendritic cells[124] underlie the etiology of asthma. However, the susceptibility of the asthmatic airway epithelium to viral infections may not be due to inherent defects in the IFN response, but rather IFN-independent antiviral responses and the influence of viral regulation on the innate immune response.

INTERACTIONS BETWEEN DIFFERENT ENVIRONMENTAL INSULTS EXACERBATE EPITHELIAL DAMAGE

Here we have discussed the mechanisms of epithelial damage by environmental insults primarily in isolation, as in vitro studies using AECs typically do. However, this does not occur in reality, where the airways are exposed to various environmental factors simultaneously or in tandem, triggering complex interactions that affect the long-term response to consequential environmental factors. For example, one of the main effects of early exposure to ETS is elevated likelihood and/or severity of respiratory viral infections.[125] This occurs as a result of damage to the structural integrity of the epithelium and also reduced immune responses of both epithelial cells and resident immune cells.[61] A number of recent studies have investigated the molecular mechanisms by which ETS may alter the immune response of AECs, making them more susceptible to RSV or HRV infection.[126-132] In vitro studies have exposed AECs to aqueous CSEs or PM over a range of times from 30 minutes to 24 hours.[126-131] Several studies found that pre-exposure of primary AECs or transformed AECs, such as BEAS-2B cells, leads to reduced antiviral chemokine production in response to HRV infection as well as elevated viral load.[128,131] A recent study[132] identified that primary AECs subjected to as little as 30 minutes of exposure to CSE reduced antiviral CXCL10 and IFN-β production and elevated susceptibility to RV-1B infection 24 hours after CSE exposure. In addition to the production of antiviral cytokines, AECs control viral infections by programmed cell death. It was demonstrated using cultured primary AECs that exposure to CSE for 2 days, followed by 1 day of RSV infection, resulted in AEC death by necrosis rather than virus-induced apoptosis. This led to elevated release of inflammatory cytokines and enhanced viral replication.[129] A reduction in innate antiviral responses by exposure to CSE has been demonstrated using nonviral stimulants such as poly I:C to treat BEAS-2B cells and human lung embryonic fibroblasts.[126]

Chemical air pollutants also interact with airborne allergens to enhance allergic sensitization in early life. For example, O_3 has been reported to increase airway responsiveness of allergic asthmatics to inhaled HDM allergen.[133] The causal link between exposure to air pollutants, allergic conditions, and the development of asthma in early childhood is still not clear due to the difficult of analyzing synergistic association in uncontrolled settings.[39] However, limited in vitro studies and mouse models have shown that the effect of allergen challenge is higher after exposure to air pollutants. It is likely that this is due to airway mucosal damage and impaired mucociliary clearance leading to elevated access of inhaled allergens to the immune system.[39]

CONCLUSION

Individuals with asthma may be predisposed to respiratory disease due to heritable traits. However, as more studies are undertaken using primary AECs, we are learning more about the role that early-life environmental insults have on shaping the long-term immune response and barrier function of the airway epithelium. The expansion of studies using ALI cultures has provided new insights into the effect of environmental factors on barrier function and wound healing. Further studies in which immune cells are co-cultured with epithelial cells grown at ALI are now required to identify how epithelial cells interact with immune cells in the establishment of long-term immunologic responses that may be dysfunctional due to exposure to environmental factors. We are also lacking a fundamental understanding of epigenetic changes within the airway epithelium that are due to early life exposures. Recent epigenetic studies have shown that exposure to air pollution and ETS can

cause long-term genetic changes in the immune response after birth and contribute to a persistent Th2 immune state in asthma.[134] The effect of environmental insults on processes such as DNA methylation, histone modification, chromatin remodeling complexes, and the role of small noncoding RNA is largely unexplored in the context of asthma. A better understanding of the effect that early-life exposure to environmental factors has on long-term respiratory health will lead to preventative interventions and better outcomes for children and adults with asthma.

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