Epithelial cells, the "switchboard" of respiratory immune defense responses: effects of air pollutants

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Summary

"*Epimmunome*", a term introduced recently by Swamy and colleagues, describes all molecules and pathways used by epithelial cells (ECs) to instruct immune cells. Today, we know that ECs are among the first sites within the human body to be exposed to pathogens (such as influenza viruses) and that the release of chemokine and cytokines by ECs is influenced by inhaled agents. The role of the ECs as a switchboard to initiate and regulate immune responses is altered through air pollutant exposure, such as ozone, tobacco smoke and diesel exhaust emissions. The details of the interplay between ECs and immune cells are not yet fully understood and need to be investigated further. Co-culture models, cell specific genetically-modified mice and the analysis of human biopsies provide great tools to gain knowledge about potential mechanisms. Increasing our understanding about the role of ECs in respiratory immunity may yield novel therapeutic targets to modulate downstream diseases.

Key words: *epithelial cells; immune response, influenza virus infection, air pollutant*

Epithelial cells initiate and regulate immune responses

Airway epithelial cells (ECs), either nasal or pulmonary, are among the first sites within the respiratory system to be directly exposed to inhaled environmental stressors, such as air pollutants, pathogens or allergens. ECs are among 40 different cell types that have been identified in the respiratory tract [1]. Some of these cell types (such as neutrophils, dendritic cells (DCs), macrophages, lymphocytes – B cells, T cells, natural killer (NK) cells, etc.) are known to be involved in innate and/or adaptive immune responses [2]. While the importance of other cell types in the context of immune responses has been recognised for many years, only recently has the role of ECs during immune responses been discussed more intensively. Swamy et al. [3] introduced the term "*epimmunome*" with the goal of identifying

"all molecules used by ECs to instruct immune cells" and pointed clearly to the crucial role of ECs during the initiation and regulation of an immune response. In addition, Vareille et al. [4] summarised the important roles of the airway epithelium in the fight against respiratory viruses. The function of airway ECs can be categorised on three different levels (fig. 1): First, ECs play an important barrier function. They provide a level of protection through their tightly organised cellular layer. Each EC is attached to its neighboring cells by cell-cell junctions, including tight junctions, adherens junctions and desmosomes [1, 5–7]. This allows the control of paracellular transport to maintain ionic gradients necessary for the directional secretion of different substances [1, 5–7]. Secondly, ECs are an important source for soluble factors such as chemokines and cy-

Abbreviations

CD Cluster of differentiation
CXCL Chemokine (C-X-C motif) ligand
DC Dendritic cell
DEP Diesel exhaust particles
EC Epithelial cell
GM-CSF Granulocyte macrophage colony-stimulating factor
GRO Growth regulated oncogene
HLA Human leukocytes antigen
ICAM Intercellular adhesion molecule
IFN Interferon
IL Interleukin
IP Interferon gamma-induced protein 10
ISG Interferon stimulated gene
ISGF3 Interferon stimulated gene factor 3
LAIV Live attenuated influenza virus
MCP Monocyte chemotactic protein
MIC MHC class I polypeptide-related sequence
MIG Monokine induced by gamma interferon
MIP Macrophage inflammatory protein
NK cell Natural killer cell
O_3 Ozone
OAS Oligoadenylate synthetases
RANTES Regulated on activation normal T cell expressed and secreted
TNF Tumor necrosis factor
ULBP UL16-binding proteins

tokines (such as interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)-α, eotaxin, RANTES (regulated on activation normal T cell expressed and secreted), monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1, interferon gamma-induced protein (IP)-10) [8–12] or lipid mediators (such as prostaglandins) [13] to attract, activate and regulate immune cells as well as neighboring ECs [8, 9, 14–16]. Thirdly, ECs are important during immune responses through the expression of receptors and ligands which directly interact, guide, activate, and regulate other immune cell types. One example is the interaction between ECs and NK cells. NK cells are effector lymphocytes which are important in the context of the early control of viral infections and tumour fighting [17–19]. They patrol the epithelium and kill virus infected cells or transformed tumour cells [20]. In addition to soluble mediators, the activation status of NK cells is controlled by the balance of various inhibitory and activation receptors interacting with ligands expressed on potential target cells [21, 22]. NKG2D, one of the most important activating receptor on NK cells, binds to various ligands, such as MHC class I polypeptide-related sequence (MIC) A, MICB, or UL16-binding proteins (ULBPs), which are expressed on stressed, but not normal airway ECs [23–26]. NKG2D - ligand binding interactions result in the killing of target cells, ultimately leading to the protection of the human body from infected or transformed cells [25].

However, more knowledge about the role of ECs during immune responses, especially how they are modified by potential stressors or resident immune cells and how they in turn affect other cells, is necessary. A detailed understanding of the role of ECs could help to develop treatments which use ECs as a therapeutic target and which address diseases downstream of ECs.

In order to further illustrate potential roles of ECs in respiratory immunity, we will use immune responses elicited by influenza infections, the subsequent immune cell type activation, and the roles of ECs as an example. We will

Figure 1

Interplay of airway epithelial cells and immune cells. Epithelial cells exposed to pathogens, allergens and air pollutants release soluble factors such as chemokines and cytokines addressing immune cells. Epithelial cells and immune cells also communicate via activating and inhibitory ligand-receptors interactions.

provide examples of how inhaled agents alter the EC response in general and especially how factors involved in the immune responses against influenza infection are modulated by air pollutants. Later, we will provide examples of tools to study the role of ECs during respiratory immune responses.

Immune responses against influenza infections – involved cell types

Influenza virions entering the human respiratory tract infect and replicate in ECs. In turn, ECs respond to the infection by synthesising and releasing various cytokines, immunoregulatory molecules and antiviral mediators [27]. Among those, RANTES, MCP-1, IL-8, IL-6 and eotaxin released by ECs recruit and activate immune cells involved in the defense and clearance of the invading virus. Type I interferons (IFNs), produced and released by ECs, induce the synthesis and activity of mediators involved in turning off the viral replication in neighboring ECs and immune cells [10, 27–29]. IFNs produced by ECs activate the JAK-STAT pathway which induces the transcription of IFN stimulated gene (ISG) factor 3 (ISGF3). ISGF3 regulates the expression of various ISGs, such as 2',5'-oligoadenylate synthetases (OAS), protein kinase R (PKR) and Mx proteins, which mediate viral RNA degradation and block virus replication [10, 30]. As indicated above, among the cell types recruited early during the infection in the upper airways are NK cells [31]. A subset of NK cells (cluster of differentiation (CD)56^{dim}CD16^{bright} cells) is considered to be more cytotoxic and releases granules containing perforin and granzymes, which induce apoptosis in influenza infected cells [32, 33]. Other subsets of NK cells (CD56^{bright}CD16^{dim} or CD56^{bright}CD16$^-$) are less cytotoxic, but secrete more cytokines, such as IFN-γ [34, 35]. However, the classification of different NK cell subsets and their functions is still being developed. Besides NK cells, macrophages and DCs have been identified in the nasal and lower respiratory submucosa [36] and are known to play important roles in the innate immune response to influenza virus infection [37]. Studies in laboratory animals have shown that macrophages are important to limit the viral spread through the phagocytosis of influenza virus-infected cells [38, 39]. Furthermore, human macrophages are known to produce cytokines which are important in the antiviral immune response and play an important role in the regulation of the adaptive immune response [40]. The professional antigen-presenting DCs – usually located underneath the airway epithelium, but above the basal membrane [41, 42] – are crucial in activating T cells. DCs present foreign antigens from infected host cells to fulfill the pivotal task of mobilising both innate and adaptive immune cells by secreting chemokines (such as chemokine (C-X-C motif) ligand (CXCL) 16, MIP-2α, MIP-2β, growth regulated oncogene (GRO) α, IP-10, RANTES) to attract neutrophils, NK cells, cytotoxic and memory T cells to the site of infection [43]. Activated DCs also up-regulate the expression of maturation receptors (such as CD80, CD86, and human leukocytes antigen (HLA)-DR) to aid in antigen presentation and provide co-stimulatory signals to T cells during influenza infection [44]. DCs migrate to peripheral lymph nodes to initiate an adaptive T cell response by activating
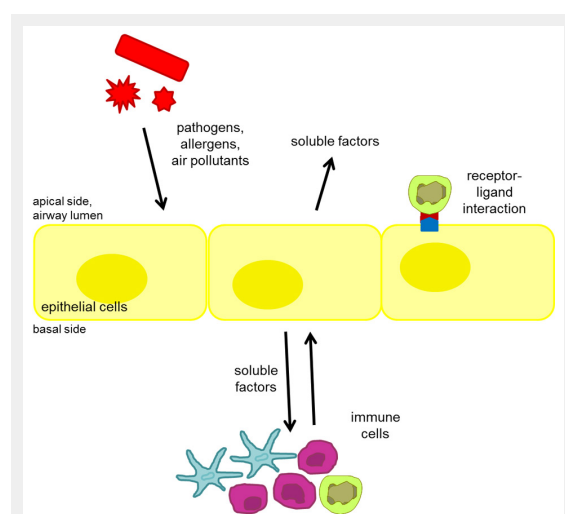
influenza specific CD4^{+} and CD8^{+} T cell clones and supporting clonal expansion of naïve T cells via autocrine IL-2 production [45]. DCs can also enhance T cell responses through production of IFN regulated chemokines such as monokine induced by gamma interferon (MIG), IP-10, and IP-11 to drive a T helper cell type 1 CD4 T cell response and expand activated cytotoxic CD8 T cell pools [45].

EC-dependent modulations of immune cells

ECs modulate **neutrophils** through the release of chemokines and cytokines which act as chemo-attractants to recruit neutrophils to the airways. Important examples are RANTES, IP-10, IL-1β, IL-6 and IL-8 [10, 27, 43, 46, 47]. Different adhesion molecules expressed on the EC surface guide the neutrophils through the EC layer after they have crossed the endothelial cell layer [48]. **Eosinophils** are also recruited to the airways and are activated, among others, by factors released by ECs, such as RANTES, eotaxin-1 and eotaxin-2 [49]. **NK cells** are influenced by ECs through the release of chemokines and cytokines and through direct cell-cell interactions. ECs are a source of IL-8, IL-12, IL-15, IL-18, RANTES, IP-10, MCP-1 and eotaxin-3, which are all important for the trafficking and activation of NK cells [8, 9, 20]. Furthermore, stressed ECs express MICA/B and ULBPs which are ligands for the important activating NK receptor NKG2D [22, 25, 50, 51]. **Macrophage** recruitment and activation is induced by chemokines and cytokines such as IL-1β, MIP-1α, MCP-1 and TNF-α, which can all be released by ECs [46, 52, 53]. ECs and macrophages also build tight junction [5] and form tight networks together with DCs [54] which allows them to communicate via direct cell-cell interactions. **Respiratory DCs** lay basolaterally to respiratory ECs in the lamina propria throughout the airways [41, 42]. DCs maintain constant contact with respiratory epithelial cells, and their projections can protrude between the tight junctions of the EC barrier to directly sample the airways for pathogenic antigens [54–56]. DCs are not only influenced by direct cell-cell interactions with ECs, but also by EC-derived mediators, such as type I IFNs [28] or MIP-3α [57]. EC-derived RANTES and IP-10 not only modulate and recruit neutrophils, eosinophils and NK cells, but also **T cells**. T cell activity is also modulated by type I IFNs released from virus-infected ECs [28].

Inhaled agents which alter EC responses

With every breath about 500 ml air and more than one million particles are inhaled, resulting in a volume of more than 10,000 liters of air and about 300 million inhaled particles per day [58]. Inhaled particles can be grouped into pathogens, allergens and air pollutants (particulate and gaseous). All three types of inhaled agents have significant effects on ECs and also on other cell types in the respiratory mucosa. In this chapter we focus on how air pollutants change the EC responses.

(Diesel) exhaust emissions

Gowdy et al. [59] showed that exposure of mice to diesel exhaust emissions causes an up-regulation of intercellular adhesion molecule (ICAM)-1, TNF-α, MIP-2, IL-6, IFN-γ, IL-13 and an enhanced neutrophil number. Similarly, exposure of respiratory ECs (cell line A549) to diesel exhaust emissions enhances the release of IL-8 and induced cytotoxicity [60]. Exposure of ECs to diesel exhaust emissions prior to infection with influenza *in vitro* [61] and exposure of human volunteers prior to inoculation of live attenuated influenza virus (LAIV) *in vivo* enhanced markers of viral replication and IFN-γ [62]. In addition, diesel exhaust emission exposure prior to infection showed an up-regulation of viral replication and IL-6 in mice [63]. In summary, diesel exhaust emissions increase the viral replication in ECs and the release of immune response specific chemokines and cytokines.

Tobacco smoke

Numerous studies have shown that exposure to tobacco smoke modulates respiratory immune responses [64, 65], many of which are initiated and regulated by airway ECs. We have recently demonstrated that human primary nasal ECs from smokers showed increased viral shedding [66] and that viral replication was higher in volunteers exposed to cigarette smoke (both active smokers and secondhand smoke exposed) than in non-smokers [64]. A study by Horvath and colleagues comparing the immune responses elicited by inoculation with LAIV vaccine showed that smoking down-regulated LAIV-induced granzyme B levels and the number of cytotoxic NK cells in nasal lavage, but not in peripheral blood [31].

Ozone (O₃)

Recent studies by Kesic et al. [67] showed enhanced viral replication in nasal ECs exposed to O_3. Several human and mouse *in vivo* and *in vitro* studies have shown that O_3 modifies factors involved in immune responses. Song et al. [68] showed increased pro-inflammatory markers and oxidative stress after acute exposure of ECs to O_3. Other studies found an enhanced release of pro-inflammatory mediators such as IL-8, MCP-1, MCP-3, RANTES, TNF-α and granulocyte macrophage colony-stimulating factor (GM-CSF) [69–73] and this effect was more pronounced in asthmatics compared to non-asthmatics [70, 71]. Interestingly, all of these chemokines are also important for the trafficking of immune cells such as NK cells [8, 9]. Exposure to hydrogen peroxide up-regulates the expression of NK cell ligands on ECs [26], suggesting that exposure to other oxidants, like O_3, has the potential to interfere with the direct cell-cell interactions between ECs and NK cell by altering the expression of NK cell ligands, such as MICA/B and ULBP3.

Tools to investigate the role of ECs

In order to gain a better understanding of the role of ECs during respiratory immune responses and how ECs could be used as targets to modulate downstream diseases, various tools can be used. ECs alone *in vitro* (either cell lines or primary cells) provide an opportunity to estimate how ECs react to a specific inhaled agent and how these reactions can be altered. To investigate how effects on ECs modulate downstream immune responses, it is important to un-

derstand cell-cell interactions with other cell types (such as fibroblasts, endothelial cell, DCs, macrophages, NK cells, mast cells, B cells, T cells, etc). Co-culture models have been shown to be a valuable tool for understanding cell-cell interactions. Horvath et al. [74] demonstrated that antiviral defense responses in DCs are different when these cells are co-cultured with ECs from non-smokers and smokers. A study by Bleck et al. [75] investigated the impact of diesel exhaust particle (DEP)-treated ECs on DCs activity using a co-culture system. Phenotypic and functional maturation of DCs was induced by co-culturing with DEP-treated ECs but not by direct stimulation of DCs with DEP treatment of the DCs. Furthermore, conditioned media from DEP-treated ECs functionally matured the DCs [75], suggesting that EC-derived soluble mediators are enhancing DC function. Another study using triple cell co-cultures consisting of the 16HBE14o⁻ bronchial EC line, monocyte-derived DCs, and monocyte-derived macrophages exposed to scooter exhaust emissions, demonstrated changes in immune cell function [76, 77].

In vivo exposure studies using cell type-specific genetically modified mice are another excellent tool to examine the role of ECs in respiratory immune responses. For example, Poynter et al. [78] generated airway EC-targeted transgenic mice expressing a mutant version of the inhibitory protein I-κBα which acts to repress the activation of the transcription factor NF-κB. In these genetically modified mice, stimulation with lipopolysaccharide resulted in a reduction of neutrophil influx, the secretion of neutrophilic chemokine MIP-2 and pro-inflammatory cytokine TNF-α compared to wildtype mice, suggesting that modifications at the level of epithelial cells mediated these changes.

Besides co-cultures and animal *in vivo* studies, human nasal or bronchial biopsies are also excellent tools to study the role of ECs and the role of specific EC factors. Hamilton and colleagues [79] used bronchial biopsies to investigate changes in tyrosine phosphorylation in the epithelium of asthmatics. They found an abnormal regulation of protein tyrosine activity in severe asthmatics and hypothesised that tyrosine kinase pathways contribute to persistent, corticosteroid-unresponsive inflammation in severe asthma. Also, several other studies used immunohistochemical analyses of human airway biopsies to address questions about the role of ECs in respiratory immune responses [80–83]. Biopsies can also be treated and stained for flow cytometry analysis which allows investigation of other endpoints than immunohistochemistry and can identify changes in immune cell types residing in the respiratory mucosa [84].

Conclusion

Respiratory ECs are among the first targets for inhaled, airborne environmental stressors, such as air pollutants, allergens or pathogens. During viral infections, ECs act as a switchboard to initiate and orchestrate downstream immune responses through the release of chemokines and cytokines, which in turn recruit and activate immune cells. The release of chemokines and cytokines can be changed by the concurrent or prior exposures to air pollutants thus altering immune responses. However, the current under-

standing about the exact roles of ECs during immune responses, how ECs are affected by inhaled agents, and how these effects in turn modulate immune responses is incomplete at best and needs to be investigated further. In particular, the interplay between ECs and respiratory or even systemic immune cells needs to be better understood so that one day we may be able to potentially use respiratory ECs as targets to modulate downstream immune responses.

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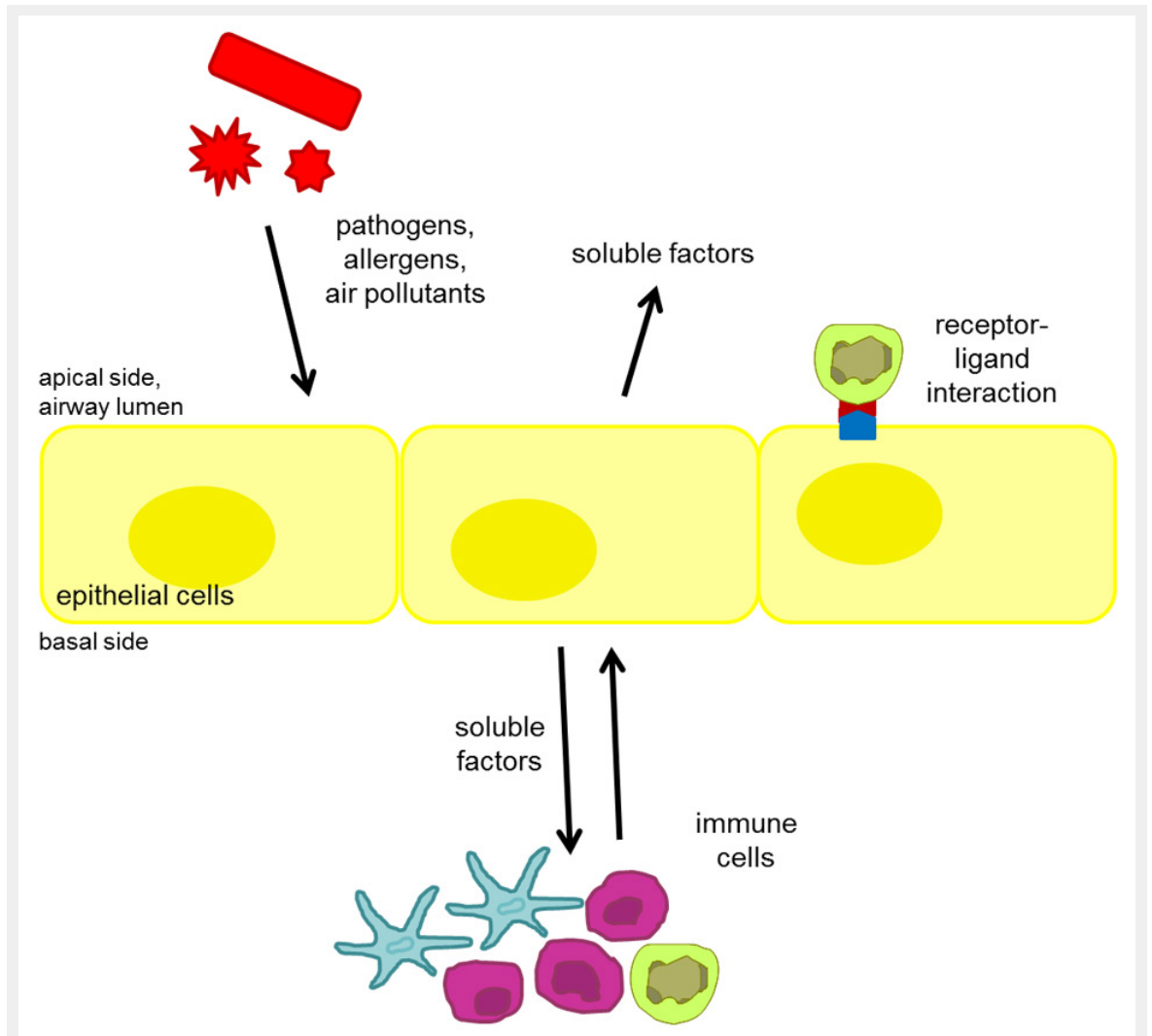
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Figures (large format)

Figure 1

Interplay of airway epithelial cells and immune cells. Epithelial cells exposed to pathogens, allergens and air pollutants release soluble factors such as chemokines and cytokines addressing immune cells. Epithelial cells and immune cells also communicate via activating and inhibitory ligand-receptors interactions.