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Current perspectives in epithelial cell injury and repair: consequences for epithelial functions

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ABSTRACT: Epithelial cells lining the airways and the respiratory compartment may, and certainly when exposed to an inflammatory milieu, display an altered functioning, which could contribute to pathophysiology of inflammatory lung/airway disease. In the present review paper, several issues that were discussed at an earlier European Respiratory Society Research Seminar on conditions that affect epithelial functioning have been recapitulated and updated. These and future studies should improve understanding of epithelial functioning and may aid recovery from disease.

KEYWORDS: Asthma, chronic obstructive pulmonary disease, epithelial, inflammation, pathophysiology

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The airways are exposed to environmental challenges such as microbial agents, toxic components and particulate material. To effectively counter these challenges, in order to maintain appropriate functioning of the lung, the airway and more distal epithelial cells constitute a major physical and complex chemical barrier that can be modulated further in response to these challenges. Over the last 15 yrs, a great deal has been learnt about these epithelial responses, using both *in vitro*, *i.e.* cell lines and primary cells, and *in vivo* approaches. Activated airway epithelial cells can modulate their direct barrier function by the release of a variety of defence molecules, such as defensins and mucins, as well as by modulating fluid transport and other physiological properties. Airway epithelial cells also release multiple mediators and express adhesion molecules that can direct and activate migratory inflammatory and immunological cells. Finally, epithelial cells interact with other resident cells, such as fibroblasts, and the underlying extracellular matrix (ECM) to restore damaged tissue. These interactions are complex and by no means unidirectional, but underline the essential contribution of epithelial cells to maintain and restore homeostasis in the airways and the respiratory compartment.

It is well known from cell biology that epithelial cells are dependent on adequate cell–cell contact and interaction with the underlying ECM for their functioning. Cellular metabolism, which depends on the availability of nutrients, is also elementary to the functioning of cells. This holds true in particular for epithelial cells that may need to compete for nutrients with micro-organisms. Changes in cell–cell and cell–matrix interactions, as well as an altered epithelial cell metabolism, are thus likely to modulate epithelial functioning. In inflammatory airway/lung diseases, such as asthma and chronic obstructive pulmonary disease (COPD), airway and lung epithelial cells are exposed, either frequently or over prolonged periods, to a range of inflammatory mediators and effector molecules from inflammatory cells (neutrophils, eosinophils, mast cells, lymphocytes). Airway and lung epithelial cells respond to many of these mediators and some inflammatory effector molecules are known to affect epithelial cell integrity and metabolism. In this context, it is also relevant that the subepithelial ECM may have changed in inflammatory lung diseases, which could influence the differentiation and functioning of the attached epithelial cells. Finally, the composition of the epithelium, which is made up of several types of epithelial cells, may also have changed in inflamed airways. This could further affect the functioning of the epithelium. Taken together, epithelial cells, and thus the epithelium in an inflamed milieu, may display altered functioning, which could contribute to the pathophysiology. It is fair to say that relatively little is known about how conditions that occur in the airways of patients with asthma or COPD affect epithelial functioning. Early studies by MATTOLI and colleagues [1–3], DEVALIA and DAVIES and their

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respective co-workers [4–6], and, more recently, by WARK *et al.* [7] clearly indicated that epithelial cells collected from the airways of asthmatics or COPD patients respond differently as compared with cells from healthy individuals. Even more so, WARK *et al.* [7] showed that these changed properties are preserved in cells which have been passaged, which is suggestive of an intrinsic difference. Whether this intrinsic difference has arisen by epithelial cell selection or by imprinting due to, for example, inflammatory stress or otherwise is unknown as yet.

In 2003, a 2-day European Respiratory Society Research Seminar entitled ''Current perspectives in epithelial cell injury and repair: consequences for epithelial functions'' took place in Paris, France. Almost 50 delegates from 10 European countries, the USA and Canada aimed to identify changes and conditions, and their modes of action that have an impact on epithelial functioning. Now, 2 yrs later, it was considered timely to give a short reappraisal of the studies presented at that meeting. The authors are also very pleased that this update is accompanied by reviews on two emerging and promising topics that were discussed at the seminar. C. Coraux and E. Puchelle describe their *in vivo* models used to study human airway epithelium repair and regeneration, which incorporates the interplay of epithelial cells with their direct surroundings, as well as with migratory cells. Another review by C.W. Frevert and P.L. Sannes deals with the matrix proteoglycans in disease and their impact on epithelial cell function, and comprises some very recent and stimulating findings.

SUMMARY AND UPDATE

During the research seminar, a large number of topics were dealt with involving various pathogenetic events. To provide an overview rather than a detailed review of all of these topics, the various presentations were clustered around pathogenetic events and attempted to summarise and update these presentations in a nut shell. Therefore, this synopsis should be regarded as a starting point for those interested in this field. The authors apologise in advance to all of their colleagues whose work was not mentioned herein.

As discussed by C. Clerici (Paris, France; also on behalf of M.A. Matthay, San Francisco, CA, USA), acute lung injury, acute respiratory distress syndrome and hypoxia may lead to alveolar leakage, which affects respiration. Alveolar fluid clearance is essential to restore respiration; however, under certain conditions, resolution of alveolar fluid may be impaired. Alveolar epithelial cells play a crucial role in alveolar fluid homeostasis. The amiloride-sensitive epithelial sodium channel (ENaC) in alveolar epithelial cells facilitates alveolar fluid clearance as it pumps out sodium, and water follows passively, probably *via* water channels, so-called ''aquaporins''. A number of conditions have been found to affect ENaC function, for example, by reducing the number of ENaC molecules (hypoxia), whereas re-oxygenation and β-agonists can reverse this process [8, 9]. The identification of channel-activating proteases (CAPs) may provide another opportunity, besides through β-agonists, to clear alveolar fluid [10]. Over the past few years, it has become evident that transforming growth factor (TGF)-β1, an inflammatory cytokine associated with fibrosis and repair, also reduces the number of alveolar epithelial ENaC molecules [11]. During more severe alveolar flooding, the alveolar epithelium may become damaged, thereby effectively reducing the number of ENaC molecules. Epidermal growth factor (EGF) and keratinocyte growth factor can promote alveolar epithelial cell migration and proliferation. I.C Davis (Birmingham, AL, USA) discussed the Balb/c RSV-A2 model to study respiratory syncytial virus-reduced alveolar fluid clearance, a process that appears related to viral replication. This process appears to be mediated primarily by uridine triphosphate, acting *via* the P2Y receptor [12]. In a recent study, HICKMAN-DAVIS *et al.* [13] showed that mycoplasma also inhibited alveolar fluid clearance, but now *via* modulation of ENaC activity by reactive oxygen-nitrogen intermediates.

Ventilation of critically ill patients is considered to exert an effect primarily on the alveolar compartment, by stretching alveolar cells, which may lead to ventilation-induced lung injury (VILI). VILI is characterised amongst others by the generation of interleukin (IL)-8 and the recruitment of neutrophils that are attracted by IL-8. S. Oudin (Geneva, Switzerland) discussed the molecular machinery within epithelial cells that senses stretching. Stretch-activated ion-channels, signalling molecules associated with focal adhesion plaques and the cytoskeleton are probably key molecular structures in the process of mechanosensing. Downstream of these structures, the three main families of mitogen-activated protein kinases, extracellular signal-regulated kinase, c-Jun N-terminal protein kinase and p38 are all activated [14, 15]. These may activate the transcriptional machinery (transcription factor nuclear factor (NF)-κB) and also, possibly, post-transcriptional processes involved in IL-8 gene expression. It is now clear that the local presence of additional pro-inflammatory stimuli, such as lipopolysaccharide (LPS), may largely amplify the VILI-induced inflammatory response, causing more severe clinical problems. How this synergism comes about is unknown.

The ECM constitutes the adhesion site for epithelial cells, as well as a reservoir of proteins and other components bound to negatively charged (sulphated) groups. The composition of the ECM is quite complex and can change during pathology, as discussed in the accompanying review by C.W. Frevert and P.L. Sannes. X. Huang (San Francisco, CA, USA) discussed the role of integrins, a large family of heterodimeric transmembrane proteins, mediating cell–matrix and cell–cell adhesion. So far, 18α- and 8β-subunits have been identified, which can give rise to at least 24 heterodimeric receptors. At least eight of these receptors are expressed by lung epithelial cells, and their role in lung injury and repair has been studied in detail using both *in vitro* and *in vivo* approaches. X. Huang discussed murine studies of the integrins α9β1, αvβ5 and αvβ6, knockouts of which display distinct phenotypes. The αvβ6 integrin is of particular interest as it appeared that the β6 knockout mice developed an exaggerated inflammation, but were protected from fibrosis [16]. The β6 knockout mice fail to locally activate TGF-β1, which normally inhibits, amongst others, matrix metalloproteinase (MMP)-12. Indeed, the β6 knockout animals develop emphysematous lesions due to MMP-12 activity. The role of αvβ6 in regulating inflammation [17] is still unclear, as is the modulation of epithelial functioning by the loss of

attachment (the tripeptide arginine-glycine-aspartic acid (RGD) in ECM components is recognised by αvβ6) to the ECM.

J. Zhu (London, UK) and W. Timens (Groningen, the Netherlands) discussed changes in the mucosa in asthma and COPD, and the expression of some key molecules at the microscopic level. The shift in the cellular composition of the epithelium in asthma and COPD, such as goblet cell hyperplasia, and that of mucous glands, may lead to important pathophysiological changes, such as enhanced mucus production and reduced mucociliary clearance. It may be envisaged that these changes perpetuate an existing pathology. Current studies focus on distinct molecules expressed by epithelial cells and in the ECM, which are known to play essential roles in lung physiology. Examples are the multi-drug resistance proteins (MDR; and MDR-related proteins like MRP1), which belong to the ATP-binding cassette transporters and that may play a role in the removal of toxic components, for example, from inhaled cigarette smoke [18]. Other molecules that are being studied are haem-oxygenase, inducible nitric oxide synthase and various mediators such as TGF-β1. In fact, the microdistribution of proteins involved in inflammation and repair may strengthen the postulated roles of these proteins in asthma and/or COPD.

In a number of presentations, the mechanisms by which exogenous factors, such as microparticles, oxidative stress and viral infection, trigger epithelial cells were discussed. B. Doornaert (Verneuil-en-Halatte, France) presented *in vitro* work on diesel exhaust particle (DEP) exposure, which is known to induce the release of epithelial pro-inflammatory mediators such as IL-8. DEP reduced integrin α3β1 and CD44 expression, which are implicated in cell–matrix interaction. DEP also reduced epithelial migration and proliferation, leading to a reduced wound closure. Interestingly, these effects were not mimicked by similarly sized carbon particles, indicating that components adsorbed to the surface of DEP, such as polycyclic aromatic hydrocarbons, may be responsible for the effects of DEP [19, 20]. Reactive oxygen species (ROS) may subject epithelial cells to oxidative stress, which is believed to occur in COPD and asthma. I. Rahman (Rochester, NY, USA) discussed how ROS may activate signalling pathways, thus leading to the activation of transcription factors (NF-κB and activator protein-1) and chromatin remodelling [21]. Current interest is in glutathione and its role in preventing oxidation of thiol groups in regulatory proteins [22]. Given their topology in the airways, epithelial cells are exposed to viruses that need to pass this barrier to enter the body. In addition, many respiratory viruses are epitheliotropic, and thus these viruses will infect and replicate in epithelial cells. Besides acute infections, epithelial cell also harbour persisting viruses or pieces of their viral genomes. S. Hayashi (Vancouver, BC, Canada) presented studies on latent adenoviral infections and, more specifically, on the role of the adenoviral protein E1A in amplifying pro-inflammatory responses in COPD. As previously shown for LPS, recent studies showed that E1A expression also amplifies epithelial responses to microparticles [23], although, for example, the IL-6 response may be reduced by cells expressing E1A [24]. E1A-expressing cells also show an increased TGF-β1 and connective tissue growth factor expression which may underlie the shift of epithelial cells in COPD to a more mesenchymal phenotype [25]. Rhinovirus infection is the most frequent trigger for exacerbations in asthma and COPD. Rhinovirus employs intercellular adhesion molecule (ICAM)-1 to enter the epithelial cell, and ICAM-1 expression on epithelial cells from asthmatics is increased. S. Whiteman (Stoke-on-Trent, UK) discussed modulation of the epithelial cell surface protein ICAM-1 by rhinovirus and interferon (IFN)-γ. Rhinovirus downregulates mRNA and protein of the soluble form of ICAM, and increases the membrane-bound form. This mechanism may promote further infection by rhinovirus. IFN-γ, a potent antiviral mediator, reduced expression of the membrane-bound form, thus limiting subsequent epithelial infection by rhinovirus [26, 27]. It is as yet unknown as to how these data relate to the recent findings by WARK *et al.* [7] that epithelial cells from asthmatics fail to go into apoptosis upon rhinovirus infection.

There are several indications that psychological and post-traumatic stresses have an effect on innate and adaptive defence mechanisms. M.H. Perdue (Hamilton, ON, Canada) provided an insight into the impact of stress on the integrity of the gastrointestinal mucosal epithelium in studies using a rat stress model. It was found that corticotropin-releasing hormone can persistently reduce mucosal barrier dysfunction, thereby reducing host defence [28, 29]. Alternatively, glucagon-like peptide-2, an intestinotrophic growth hormone, improves intestinal epithelial barrier function [30]. The impact of hormones on the gastrointestinal mucosa put forward a strong case for performing similar studies on the respiratory tract.

Epithelial repair mechanisms and *in vivo* models studying these mechanisms were discussed by E. Puchelle (Reims, France), and are reviewed in far more detail in the accompanying review. EGF is one of the mediators involved in epithelial repair. However, work presented by S. Puddicombe highlighted the role of the EGF receptor as a means to amplify IL-8 responses by bronchial epithelium in asthma [31]. Recent studies have implicated enhanced tyrosine kinase activity upon exposure to EGF in epithelial cells from severe asthmatics [32].

Two presentations dealt with post-transcriptional regulation of gene expression. S. Stamm (Erlangen, Germany) presented studies on the common process of alternative splicing, as consequence of which proteins can display alternative functions, which could lead to physiologically meaningful changes [33, 34]. Interestingly, evidence is accumulating that exogenous stimuli may regulate alternative splicing. For example, the switch from soluble to membrane-bound ICAM-1 upon rhinovirus infection described earlier is likely to be due to alternative splicing, as these changes were also seen at the mRNA level. Finally, in place of T. Henics, R. Lutter discussed how exogenous factors relevant in asthma and COPD (IFN-γ, IL-17, a distorted cytoskeleton, viral infection, metabolic stress) reduce degradation of the normally short-lived mRNAs from response genes, such as IL-8 and IL-6. Stabilisation of these mRNAs has a large impact on the production of the encoded protein, as reflected by largely amplified dose–response curves [35, 36] for many stimuli, as well as by a prolonged production. Although epithelial IL-8 and IL-6 production are normally dampened by corticosteroids, under certain conditions that

lead to stabilisation of the encoding mRNAs, responses become insensitive to corticosteroids. Recent studies have indicated that some of the aforementioned exogenous factors directly modulate translation.

CONCLUSION

From these studies it is clear that epithelial functioning is modulated by a host of endogenous and exogenous factors that are manifest in lung diseases. EGF and transforming growth factor-β1 are typical examples of mediators present in inflammatory airway/lung disease affecting several epithelial functions. Improving our understanding of epithelial functioning at normal and pathological conditions, both *in vitro* and *in vivo*, may provide means to intervene with epithelial functions, which ultimately may aid recovery from disease.

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