REVIEW

Modulation of airway responsiveness by the airway epithelium in humans: putative mechanisms

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Introduction

The airway epithelium forms the interface between the respiratory system and the external environment and consists of ciliated and non-ciliated cells tightly attached to each other and to the basement membrane [1]. In bronchial asthma, areas of airway epithelium become damaged and the degree of epithelial damage correlates with the level of bronchial responsiveness [2,3]. Inflammatory cells present in the asthmatic airway mucosa may contribute to the epithelial damage by the production of oxidants, proteases and cationic proteins [1,3–5]. In addition, airway epithelium may be disrupted by an increased subepithelial hydrostatic pressure caused by oedema of the inflamed airway wall [6]. Several mechanisms have been proposed to explain the relationship between epithelial damage and airway hyperresponsiveness. These include, firstly, a reduced production of epithelium-derived relaxing factors (EpDRF) [7] and secondly, a decreased metabolization of bronchoconstricting mediators and neurotransmitters by the damaged epithelial cells [8]. Thirdly, loss of epithelial integrity may increase airway permeability and provide easy access of bronchoactive mediators to the airway smooth muscle [9]. Finally, epithelial damage may expose intra-epithelial sensory (peptidergic) nerves. Excitation of these sensory nerves by inflammatory mediators may lead to a local reflex bronchoconstriction via release of neuropeptides or tachykinins [10]. The above-mentioned hypothetical mechanisms are schematically shown in Figure 1 and will be discussed in some detail in the present review.

Production of bronchoactive mediators by the airway epithelium

After the discovery of endothelium-derived relaxing factor (EDRF, now identified as nitric oxide [11]) in blood vessels it was hypothesized that a similar factor might be produced by the airway epithelium. In asthma,

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this epithelium-derived relaxing factor (EpDRF) might not be produced in sufficient amounts to keep the airway smooth muscle in a relaxed state and thereby contribute to bronchoconstriction. Several candidates for EpDRF have been put forward including arachidonic acid metabolites [7] and, more recently, NO [12,13].

In vitro, human airway epithelial cells are indeed able to metabolize arachidonic acid to both cyclooxygenase and lipoxygenase products [14]. However, the cyclooxygenase products thromboxane A_2 (TxA_2), prostaglandin D_2 (PGD_2), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) constrict human airway smooth muscle both *in vivo* and *in vitro* through activation of the thromboxane prostanoid (TP)-receptor [15,16]. Prostaglandin E_2 (PGE_2) and prostaglandin I_2 (PGI_2, prostacyclin) may relax or contract airway smooth muscle depending on which prostanoid receptor subtype is involved [16].

5-Lipoxygenase is the most important enzyme of the lipoxygenase pathway. It catalyzes the formation of the unstable leukotriene LTA_4 which is further converted to LTC_4, LTD_4, and LTE_4 [14]. Leukotrienes, especially LTC_4 and LTD_4, are potent constrictors of airway and vascular smooth muscle and increase microvascular permeability and mucus secretion [17]. Because all lipoxygenase products and most cyclooxygenase products contrast human airways it is not likely that they act as EpDRF. Indeed, human isolated airways relax to endogenous prostanoids only in the presence of a TP-receptor blocking drug [18].

The highly reactive gas nitric oxide (NO) is formed from L-arginine by the enzyme NO-synthase (NOS) which has been demonstrated in human airway epithelium [19]. NO relaxes smooth muscle through activation of soluble guanylate cyclase and elevation of intracellular cyclic guanosine monophosphate (cGMP) [19]. Recently, it was suggested that analogous to the vascular endothelium, the airway epithelium produces NO that may act as EpDRF [12,13]. In the blood vessels, the NO-producing endothelium directly lines vascular smooth muscle. Epithelium-derived NO, however, has to pass a dense subepithelial vascular plexus to reach airway smooth muscle.
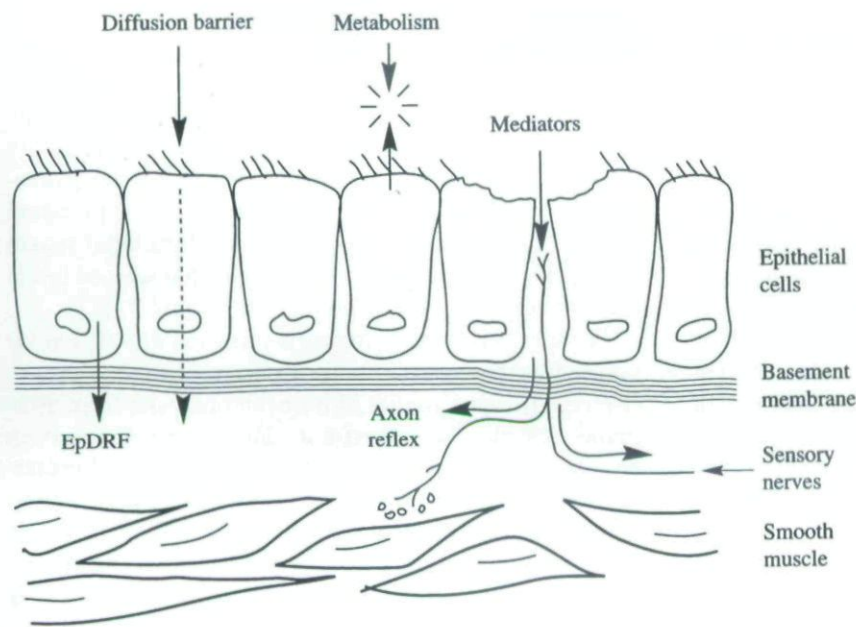
Fig. 1. Putative mechanisms explaining the relationship between epithelial damage and airway hyperresponsiveness. EpDRF = epithelium-derived relaxing factor.

Because NO eagerly binds to haemoglobin [11] and relaxes airway smooth muscle only at high concentrations [20], it is not likely that *in vivo* epithelial NO molecules will reach airway smooth muscle in sufficient amounts to produce airway smooth muscle relaxation. Indeed, in healthy humans, inhaled NO produces a potent vasodilation of pulmonary vessels [21] but only a slight bronchodilation [22]. It can be hypothesized, therefore, that products released by the airway epithelium may have a more important modulatory role on the airway mucosal bloodflow than on airway smooth muscle responsiveness and this may be an important determinant of airway responsiveness as well.

Metabolic functions of the airway epithelium

The airway epithelium not only provides a physical barrier restricting access of inhaled noxious stimuli but also actively defends the underlying submucosa: bronchoactive peptides like tachykinins and kinins are degraded by epithelial membrane-bound neutral endopeptidase (NEP) and angiotensin-converting enzyme (ACE) [8,10]. Similarly, the neurotransmitter acetylcholine and the inflammatory mediator histamine are inactivated by epithelium-derived acetylcholinesterase and N-methyltransferase, respectively [23,24]. Oxidants, either inhaled or produced by inflammatory cells, are inactivated by antioxidants in the epithelial lining fluid and by the intracellular enzymes superoxide dismutase, catalase and glutathione peroxidase [25,26]. Airway epithelial cells may also contribute to host defence by modulating the local mucosal immune response: Inflammatory cells are recruited into the airway lumen by chemotactic mediators and cytokines released by epithelial cells [27,28]. Furthermore, epithelial cells are able to express specific receptors called adhesion molecules on their surface which enable them to interact with migrating inflammatory cells [27,28].

In experimental settings removal of the epithelium or addition of specific enzyme inhibitors causes hyperresponsiveness of the airway smooth muscle to inflammatory mediators, and neurotransmitters which suggests an important metabolic role of the airway epithelium [8,18,23]. This is supported by the finding that viral infections and environmental pollutants such as cigarette smoke and toluene diisocyanate decrease epithelial NEP activity and increase airway responsiveness [29].

However, airway hyperresponsiveness cannot be explained by impaired metabolic function of the damaged epithelium alone. For instance, NEP has also been demonstrated on sub-epithelial tissues both functionally and by immunohistochemistry [18,30]. Moreover, although asthmatic patients are hyperresponsive to inhaled neuropeptides, no evidence for reduction of NEP activity was found in these patients [31,32] perhaps because there are sufficient non-epithelial sources of NEP in the airways. Thus, although the airway epithelium has the potential to act as a metabolic site where bronchoconstrictive stimuli are inactivated, evidence that this metabolic function deteriorates in asthma is lacking.

Epithelium as a diffusion barrier

The airway epithelium forms a barrier against leakage of water and solutes into the airways and prevents penetration of inhaled material to the airway interstitium [6]. This barrier function of the epithelium is achieved through several adhesive mechanisms which are shown in Figure 2 [1,33]. The desmosome (macula adherens) and the intermediate junction (zonula adherens) maintain cell-to-cell adhesion. Intermediate junctions form a ring-like adhesive mechanism around the cell to which adhesion molecules have been localized [1,33]. Hemidesmosomes consist of peptides called integrins and anchor the basal cells to the basement membrane. The tight junction (zonula occludens) is a narrow belt-like structure of unknown composition surrounding each cell at the apical pole. Tight junctions are considered as a major component of the epithelial barrier and regulate paracellular transport of large and hydrophilic molecules [33]. The permeability of tight junctions can be modulated by proteases, cytokines, eosinophil- and neutrophil-derived proteins and bacterial products [34]. Because of their important function, evidence of damage to tight junctions has been sought in disease states like pulmonary oedema and asthma. In asthmatic patients, increased airway permeability to inhaled hydrophilic tracer molecules was found [35] and electronmicroscopical studies of bronchial biopsies from asthmatic patients revealed opening of tight junctions and widening of intercellular spaces in the airway epithelium [3]. Moreover, the frequency of opening of tight junctions

correlated with eosinophil infiltration and the degree of bronchial responsiveness suggesting a causal relationship [3]. These observations are supported by studies of human airways *in vitro*: luminal exposure of human airways to oxidants or cationic proteins increased both airway responsiveness and permeability to the hydrophilic drugs (Figure 3) [36,37]. Histological examination of these airways revealed widening of intercellular spaces and opening of tight junctions in areas that seemed intact on light microscopical examination (Figure 4) [37].

There are, however, arguments against loss of barrier function as the main mechanism of bronchial hyperresponsiveness. In some studies of bronchial biopsies from asthmatic patients no structural damage to the airway epithelium was found although the patients were hyperresponsive to inhaled methacholine [38]. Similarly, airway hyperresponsiveness in laboratory animals exposed to cationic proteins was caused by the release of bradykinin and no evidence for epithelial damage was found [39]. Furthermore, although bacterial toxins may increase tight junction permeability [34], airway hyperresponsiveness is not typically found during bacterial pneumonia.

Viral infections, on the other hand, induce airway hyperresponsiveness [40] and although this has been attributed to virus-induced epithelial damage, recent evidence suggests that airway hyperresponsiveness after viral infections may be related to a deficiency in NO [41]. Finally, studies in guinea-pigs *in vivo* have shown that a denuded basement membrane is soon covered by a plasma-derived gel followed by flattening and migration of secretory and ciliated epithelial cells over the denuded
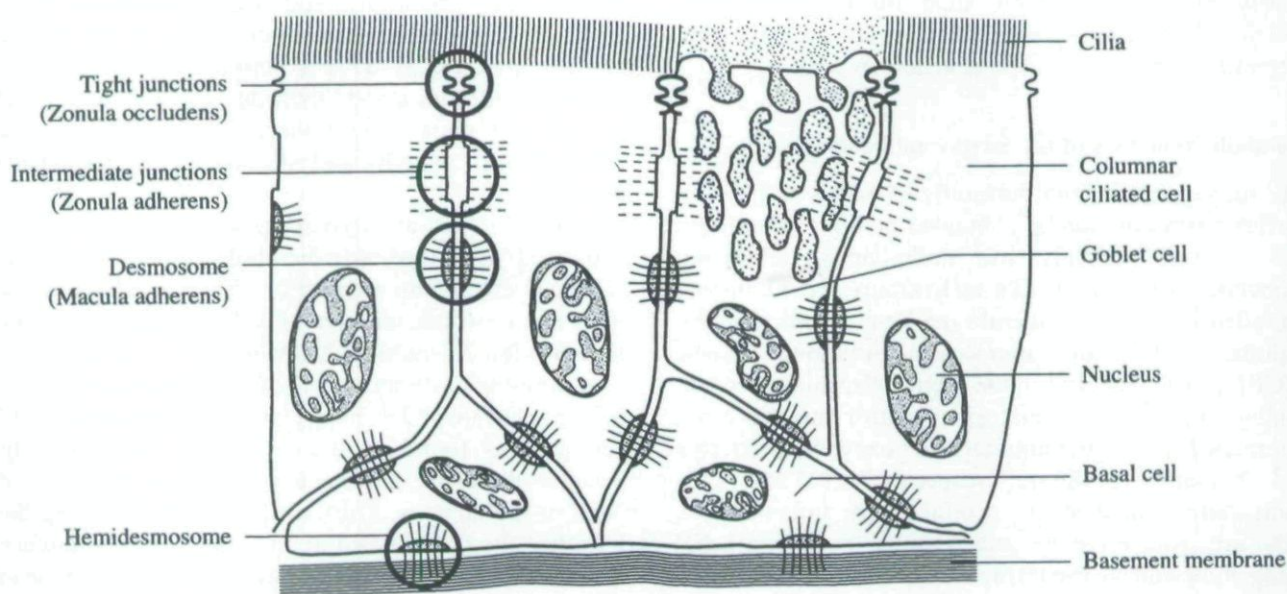

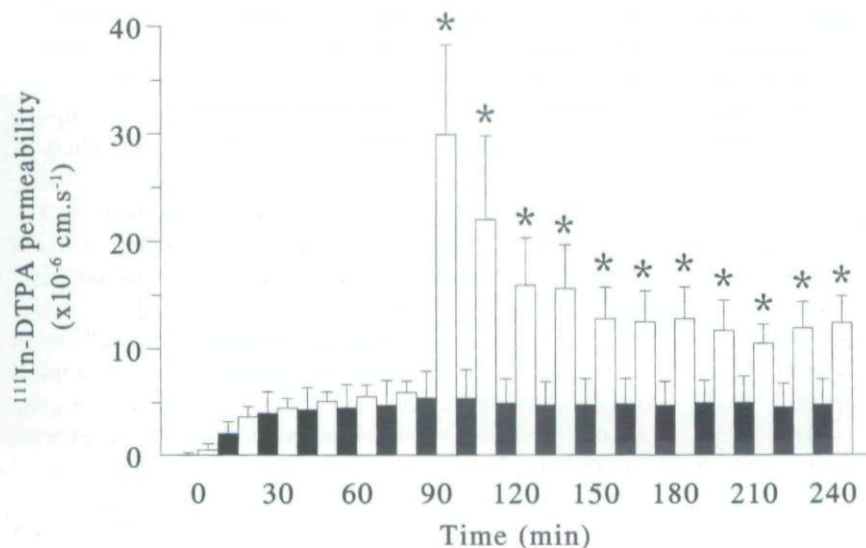**Fig. 2.** Schematic representation of the adhesion of the bronchial epithelium.

Fig. 3. Time courses of the effect of luminally applied hydrogen peroxide (H_2O_2) on permeability of human isolated airways to ^{111}In-DTPA ($n = 10$). □ = exposure to H_2O_2 110 mmol/l at T = 90 min for 15 min. ■ = controls not exposed to H_2O_2, *$P < 0.05$ compared with controls. Reproduced with permission from Hulsmann *et al.* (1996) [37].

area [6]. Thus, *in vivo*, the barrier function of the epithelium may be maintained or restored rapidly after epithelial damage. However, these repair mechanisms have not been studied in human airways.

Exposure of intraepithelial sensory nerves with release of tachykinins

In animal and human airways, superficial unmyelinated sensory nerves (C-fibres or peptidergic nerves) have been identified that terminate in the airway epithelium. These nerves contain sensory neuropeptides such as the tachykinins substance P (SP) and neurokinin A (NKA) which may be the neurotransmitters of the excitatory non-adrenergic, noncholinergic (NANC) nervous system [10,42]. In the guinea pig, excitation of these nerve fibres by chemical and physical stimuli may produce retrograde (antidromic) impulses that result in local release of tachykinins (Figure 1) [43–45].
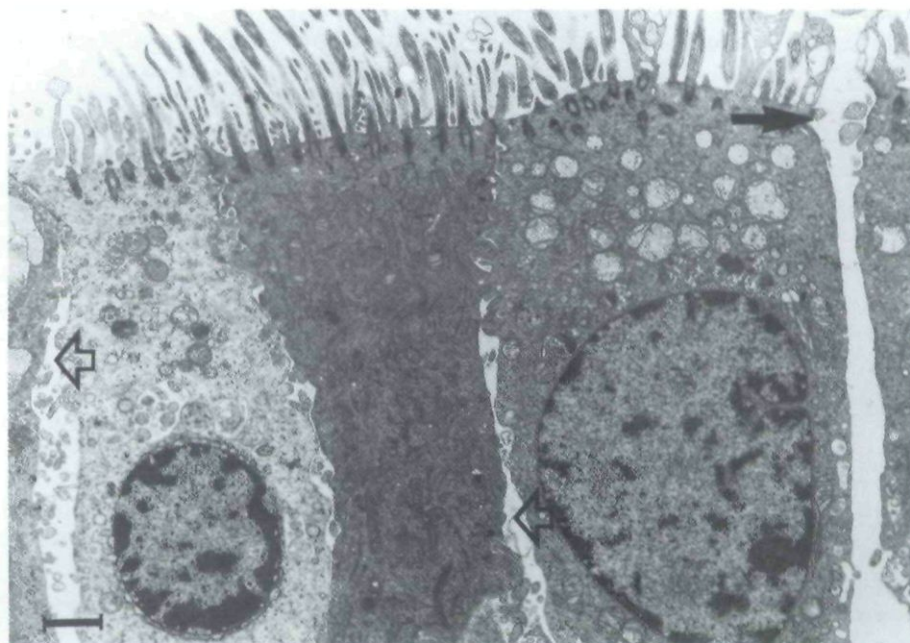

Fig. 4. Transmission electron photomicrograph of human bronchial epithelium after exposure to 100 mmol/l H_2O_2 for 15 min. Open arrows indicate widening of intercellular spaces; closed arrows depict an opened tight junction area, bar = 1 μm. Reproduced with permission from Hulsmann *et al.* (1996) [37].

Tachykinins induce airway smooth muscle contraction, mucus secretion, vascular hyperpermeability and dilation of tracheal and bronchial blood vessels, and stimulate and attract inflammatory cells [10,42–45]. Because of these effects, called 'neurogenic inflammation', tachykinins have been implicated in the pathophysiology of asthma. Compared to guinea-pig airways, however, human airways contain much less tachykinin-containing nerve fibres [45] and less tachykinin receptors [46] and this would suggest that in human airways neurogenic inflammation may be less relevant. Recently, however, an increased SP-immunoreactivity in bronchoalveolar fluid and sputum has been reported in asthmatics [47,48]. Furthermore, inhalation of a tachykinin receptor antagonist partly prevented bronchoconstriction induced by the C-fibre-stimulating inflammatory mediator bradykinin [49].

Although these studies suggest that endogenous tachykinins are released during an asthmatic attack, they do not prove that these tachykinins are released by nerves and not, for instance, by inflammatory cells present in the airways. Therefore, we investigated the effect of the selective C-fibre-stimulating drug capsaicin [50] on human isolated airways. Capsaicin caused small contractile responses in human airways. In addition, capsaicin induced the release of the tachykinins SP and NKA by these airway preparations, which could be inhibited by the neural conductance blocker tetrodotoxin (TTX) [51]. This indicates that the tachykinins were indeed released by nerve fibres, probably through a local axon reflex mechanism. Although in our experiments with human isolated airways these local axon reflexes did not produce important bronchoconstriction, *in vivo* local axon reflexes may produce other features of 'neurogenic inflammation' such as tracheobronchial vasodilatation, and inflammation and oedema of the airway mucosa. These processes may increase airway wall thickness and thereby contribute to airway narrowing [52,53]. Further studies are necessary to elucidate the relevance of local axon reflexes *in vivo* in asthmatic patients.

Conclusions

From the present experimental evidence, the relationship between airway epithelial damage and airway hyperresponsiveness cannot be easily attributed to one of the putative mechanisms mentioned in our introduction. Firstly, the airway epithelium releases many substances that may influence airway smooth muscle tone, but the functional importance of an epithelium-derived relaxing factor analogous to EDRF/NO in the vasculature is still not confirmed in humans. Epithelium-derived factors may indirectly contribute to airway narrowing by modulating airway mucosal bloodflow and enhancing inflammation. Further studies, both *in vitro* and *in vivo*, are required to confirm this hypothesis.

Secondly, many studies have shown that the airway epithelium degrades bronchoactive substances. There is, however, no conclusive evidence for a diminished metabolic function of the airway epithelium in asthma. The third hypothetical mechanism is supported by the demonstration of increased permeability of human asthmatic airways *in vivo*. Furthermore, epithelial damage increases both airway responsiveness and permeability of isolated human airways. Studies in laboratory animals suggest maintainence or rapid repair of the barrier function after epithelial damage *in vivo* and this process of epithelial repair should also be investigated in human airways *in vivo*.

Finally, recent evidence suggests that local axon reflex mechanisms which release tachykinins are present in human airways. The functional relevance of these axon reflexes for asthmatic airway inflammation and narrowing *in vivo* remains to be elucidated. From the data reviewed, it is evident that the human airway epithelium is a source of many active substances that may influence airway smooth muscle tone and airway reactivity directly or indirectly. The precise mechanisms and clinical relevance of these putative factors require further investigation.

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