

EDITORIAL

Increased permeability of asthmatic epithelial cells to pollutants. Does this mean that they are intrinsically abnormal?

Introduction

In this issue of the journal, Bayram and coworkers describe the effects of ozone (O₃) and nitrogen dioxide (NO₂) exposure of the permeability of primary cultures of human bronchial epithelial cells (HBEC) derived from normal subjects and those with asthma [1]. The authors demonstrate that exposure of normal HBEC to O₃ or NO₂ did not affect movement of ¹⁴C-BSA or electrical resistance. In contrast, permeability of monolayers to ¹⁴C-BSA was significantly increased and electrical resistance decreased in cultures of asthmatic HBEC following exposure to these oxidizing agents. The authors conclude that asthmatic HBEC are more susceptible to these pollutants than normal subjects. However, whether the enhanced sensitivity to these agents contributes to, or is a product of, the condition is unknown.

The importance of the bronchial epithelium

There can be little doubt in regard to the importance of the epithelium in controlling many of the responses seen in respiratory diseases in general and asthma in particular [2]. It constitutes the interface between the external environment and the internal milieu of the lung and as such is the point of first contact for respirable particles, respiratory viruses and airborne allergens. Under normal circumstances, the epithelium forms a highly regulated and tight barrier through the expression of tight junctions. The contiguous expression of tight junction protein complexes at the apical perimeter of epithelial cells creates adhesive structures that form tight seals with neighbouring cells, denying foreign particles paracellular access to the internal lung environment. Occludins and claudins are transmembrane adhesion proteins of tight junctions, whereas ZO-1 is one of several intracellular proteins associated with tight junction plaques. Structural integrity also depends on other cell–cell and cell–matrix interactions such as adherens junctions (E, N and P cadherens), desmosomes and hemidesmosomes [3].

In asthma, the airway epithelium is abnormal. There are structural changes involving separation of cells from the basement membrane and each other [4]. Morphological changes that include squamous metaplasia and goblet cell transdifferentiation and functional changes including an increased expression and release of pro-inflammatory cytokines, growth factors and mediators. As a consequence, epithelial damage in asthma may disrupt airway homeostasis at a number of levels, by: (1) contributing to airway narrowing via impaired secretion of

inhibitory substances; (2) disrupting fluid balance and plasma exudation, resulting in oedema and changes in the rheological properties of mucus leading to impaired mucociliary clearance; (3) releasing factors involved in the recruitment and activation of inflammatory cells and; (4) altering antigen presentation, leading to aberrant cytokine signals.

Breaking through the epithelial barriers

If, under normal conditions the epithelium forms an almost impermeable barrier, how then do airborne proteins and other noxious stimuli gain access to subepithelial structures?

Allergens with proteolytic activity such as Der p 1 from the house dust mite disrupt mucosal integrity and do so by breaking down intracellular tight junction. This is manifested by increases in epithelial permeability but moreover may provide proteolytic allergens with a mechanism for enhanced antigen presentation to dendritic cells. The mechanism involves direct extracellular cleavage of occludins and claudins [5]. In a series of elegant studies, Wan and colleagues used two-photon molecular excitation microscopy to quantify tight junction proteins *in situ* and were able to correlate time-dependent decreases of occludin and ZO-1 with increases in epithelial permeability, following exposure to Der p 1 complexes [6]. Importantly, epithelial permeability is altered in a non-specific manner by house dust mite allergens. The increased flux of ¹⁴C-Mannitol suggests that only paracellular pathways were disrupted. Markers of cell damage or death such as lactate dehydrogenase or trypan blue exclusion were normal, highlighting that these effects were specifically related to changes in tight junction integrity. Disruption of tight junctions then triggers intracellular processing and indirect proteolysis of tight junction protein.

Environmental pollutants

In vivo assessment

Pollutants such as O₃, NO₂, and to a lesser extent sulphur dioxide (SO₂) and formaldehyde, are important inhaled oxidant pollutants found in indoor and outdoor environments. Their toxic effects are a function of the concentration and duration of exposure although it appears that NO₂ is less toxic than O₃ at identical concentrations [7]. Recently much attention has

focused on determining whether ozone-induced responses are magnified in subjects 'at risk', including asthmatics, smokers and patients with COPD. Studies involving mild asthmatics have demonstrated that O₃ in concentrations as little as 200 p.p.b. produces significant cell damage as well as markers of permeability [8]. However, direct histopathological evidence of damage to tight junctions is rare. Instead, increased concentrations of proteins within the BAL fluid is often taken as indirect evidence of a breakdown in junctional integrity. However, the utility of this index is limited since it does not discriminate between increases in total protein due to perturbations of vascular-epithelial barrier vs. those that are attributable to cell lysis or increased mucous production [9]. More recently, the determination of serum concentrations of Clara cell protein has been used to assess altered epithelial cell permeability. Under normal conditions, Clara cell protein is found primarily at the mucosal surface of the airway, but when the epithelial barrier is breached, it appears in the blood [10].

In vitro studies

The present study by Bayram and coworkers provides the first evidence that asthmatic epithelial cells develop increased permeability in response to O₃ and NO₂ compared with cells isolated from healthy controls. The observation that baseline measurements of electrical resistance and transepithelial ¹⁴C-BSA flux were similar in both normal and asthmatic cultures suggests that asthmatic cells can form 'normal' tight junction complexes. As Bayram and colleagues point out, the increased permeability seen in the asthmatic cells may demonstrate that the asthmatic cells are more sensitive to the effects of these stimuli. They argue that their results are similar to other findings within their laboratory that have shown that O₃ and NO₂ decreased electrical resistance of cultures of nasal epithelial cells obtained from rhinitics during pollen season, although this effect was not observed when cultures were taken from rhinitics out of pollen season. However, an equally cogent argument can be made for these diseases (and therefore epithelial cells) being dissimilar, since the asthmatic HBEC cultures used in the current study were obtained when the subjects were clinically stable.

Mechanism of action

Although the current study and previous work by this group have elegantly shown that asthmatic HBEC are far more sensitive to the toxic effects of O₃ and NO₂ compared to normal cells, the mechanisms by which these agents increase permeability are still unknown. As mentioned previously, direct histological assessment of tight junction disruption following exposure to O₃ or NO₂ is rare [11]. In the *in vivo* situation, it is easy to see that these pollutants activate epithelial cells to release chemokines which attract and activate circulating inflammatory cells such as eosinophils and neutrophils. These cells then release a barrage of proteolytic enzymes to enable them to travel between cells. Indeed, Newson and coworkers [8] found that exposure to O₃ caused an increase in myeloperoxidase and eosinophil cationic protein levels in induced sputum from mild asthmatic subjects. Obviously, this does not provide an adequate explanation to results obtained *in vitro*. One clue comes from studies examining the effects of cigarette smoke. Exposure to cigarette

smoke has been shown to induce a reversible, concentration-dependent increase in the permeability of a monolayer of alveolar epithelial cells, which was associated with a loss in intracellular antioxidants such as glutathione [12]. This led MacNee and colleagues to propose that oxidant stress (such as O₃)-induced changes in intracellular GSH levels was the mechanism behind the increase in permeability [13]. It is also widely accepted that O₃ causes peroxidation of membrane lipids. The resultant formation of isoprostanes may exacerbate the inflammatory response by causing direct cell damage, but there is no evidence to date that this contributes to changes in permeability. An additional, but possibly important factor is that O₃ has been shown to inhibit the activity of α 1-anti-protease inhibitor [9].

The choice of *in vitro* models to assess changes in permeability.

To adequately determine cellular mechanisms, the choice of an *in vitro* model is of critical importance. As is demonstrated by Bayram and colleagues, the use of primary cell lines should first and foremost be the model of choice, although this is not always feasible or practical, particularly for diseased cells. However, even the use of primary cultures requires caution, particularly if the tissue is taken from a patient with significant airway disease or if they have been chronic smokers. Alternatively, cell lines such as Calu-3 or 16HBE14o⁻ express tight junctions, adherens junctions and desmosomes enabling them to form excellent functional barriers and thus would be suitable models of 'normal' HBEC [14]. In contrast, other immortalized cell lines such as A549 and BEAS-2B do not appear to express junctional proteins as such, and are therefore inherently 'leaky'. Other non-respiratory cell lines such as Madin darby canine kidney cells also express tight junctions, intermediate junctions and desmosomes and would also be suitable models [5].

The Big Question – are asthmatic epithelial cells intrinsically different to normal cells?

The report by Bayram and colleagues provides compelling *in vitro* evidence to show that asthmatic HBEC may be intrinsically different to normal cells. Similar studies have shown complementary responses of HBEC to diesel exhaust [15]. Evidence that the epithelium of asthmatic patients is structurally abnormal *in vivo* is accumulating. Asthmatic epithelial cells also express greater levels of activation markers and release greater amounts of pro-inflammatory cytokines into BAL. Importantly, it appears that asthmatic epithelial cells retain this abnormality in culture and it is maintained with serial passaging [15, 16]. However, as the authors themselves conclude, whether the differences seen in asthmatic HBEC are a contributing factor in the development of the disease or secondary to it, is unknown. One way to explore molecular mechanisms underlying this question is through the use of gene array technology. Such an approach has recently been taken to analyse gene expression in the nasal mucosa of normal subjects and those with allergic rhinitis [17]. Transcriptional analysis of asthmatic

and normal HBEC should help in developing a better understanding of the importance of this cell in asthma and ultimately allow new therapeutic opportunities to be realised.

References

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