

Defective function at the epithelial junction: A novel therapeutic frontier in asthma?

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The airway epithelium forms a highly regulated physical barrier that normally prevents invasion of inhaled pathogens and allergens from the airway lumen. Beyond being a simple physical barrier, the airway epithelium is now known to be a prominent regulator of the immune response to environmental triggers. Epithelial barrier function is maintained by apposed cell-cell contacts consisting of tight junctions (TJs), gap junctions, adherens junctions, and desmosomes. As far back as the early 1990s, ultrastructure studies demonstrated that the airway epithelium of the asthmatic patient has fewer intercellular adhesion junction complexes compared with that of the nonasthmatic patient, but the molecular mechanisms underlying these structural differences were not described. Recent studies have now identified that the asthmatic epithelium displays reduced and altered expression of the adhesion proteins E-cadherin and zonula occludens 1 (ZO-1).^{1,2} This phenomenon is not only confined to the lower airways because disrupted desmosome formation has also been shown in nasal polyps from asthmatic children.³ The proposed mechanism for this epithelial barrier damage was originally thought to occur because of downregulation of junctional proteins indirectly through inflammatory mediator release or directly through cleavage by allergens.^{4,5} The presumed consequences of this disrupted epithelial barrier function would be increased access of inhaled allergens to antigen-presenting cells in the submucosa and spasmogenic agonists reaching the smooth muscle.⁶

Several studies have attempted to model the effect of disrupting intercellular adhesions both *in vitro* and *in vivo*. Exposures of airway epithelial cells *in vitro* to the dust mite allergen Der p 1,^{4,7} histamine,⁸ or pollen from giant ragweed, white birch, Kentucky blue grass and Easter lily⁹ have all been shown to degrade or disrupt airway epithelial intercellular adhesions *in vitro*. Sajjan et al¹⁰ infected a human airway epithelial cell line with rhinovirus

and demonstrated reduced transepithelial resistance, increased permeability to inulin, and immunohistochemical evidence of a loss of ZO-1 from TJs. Similar effects have been shown for inhalation of the cationic protein poly-L-lysine in naive mice *in vivo*.⁶ Park et al¹¹ showed that inhalation of ovalbumin in sensitized mice also disrupted TJs, as well as connexin 37, in lung epithelial cells. The relatively few studies of these junctional proteins in human asthma have reproducibly shown that expression of ZO-1 and E-cadherin are downregulated in the asthmatic epithelium, suggesting a broad defect in adhesion mechanisms.^{1,2,12} However, the functional consequences of these differences have not been explored in detail.

In this issue of the *Journal*, Xiao et al¹³ report irregular intracellular staining of the epithelial TJ proteins ZO-1 and occludin in bronchial biopsy specimens from patients with mild, moderate, and severe asthma compared with that seen in healthy subjects, corroborating and extending these earlier findings. Importantly, when epithelial cells were studied as differentiated cultures, expression of ZO-1 and occludin remained downregulated in cultures from asthmatic patients. Similar findings were recently shown for E-cadherin,² suggesting that intrinsic abnormalities underlie the blunted expression of these junctional proteins. Because the authors did not observe changes in mRNA expression for these proteins, they suggest that these alterations in ZO-1 and occludin expression are due to posttranslational modifications.

Importantly, Xiao et al¹³ demonstrate that aberrant expression of ZO-1 and occludin in cultures from asthmatic patients is associated with a modest but statistically significant reduction in transepithelial electrical resistance (TER) when compared with that seen in nonasthmatic control subjects. After further analysis, the authors showed that the reduced TER is only present in epithelial cells from patients with severe asthma but is not modified by steroid use. These changes in TER were inversely correlated with macromolecular permeability, particularly to small-molecular-weight dextran molecules (<4 kD).

Most importantly, the authors extend previous studies that have highlighted the role of epidermal growth factor (EGF) in restoring barrier function after epithelial injury. Specifically, they demonstrate that apically applied EGF, modeling topical delivery not only enhances baseline expression of ZO-1 and occludin, leading to functionally enhanced TER in asthmatic epithelial cultures, but also enhances TER when applied apically after cigarette smoke extract exposure. Intriguingly, EGF applied apically enhanced TJ formation in cells from asthmatic patients without significantly increasing proliferation or goblet cell number. In contrast, EGF applied basolaterally, modeling systemic delivery, had no effect on proliferation or TJ formation.

Several facets of the design of this study are worth comment. First, Xiao et al¹³ used biopsy specimens and primary cells from a large number of very well phenotyped asthmatic (n = 43) and

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nonasthmatic ($n = 40$) adults. However, it would be of interest and significance to compare these findings with those in cells obtained from children with asthma of varying severity to further understand whether this phenomenon appears early in disease pathogenesis.

Second, given the recent findings relating to the keratinocyte junctional protein filaggrin and its association with the development of allergic disease,¹⁴ inclusion of an atopic nonasthmatic control group would have potentially provided important information regarding the effect of atopy on expression of TJ complexes.

Third, although the study focuses on TJs, several components of the TJ complex, including claudins, ZO-2, and ZO-3, for example, were not assessed, and therefore it is unknown whether future therapeutic strategies should be focused on ZO-1 and occludin alone.

Finally, the choice of growth factor deserves mention. The authors propose that EGF receptors on basal cells might trigger proliferation, whereas EGF receptors on more differentiated apical cells might promote TJ formation. However, no mechanism was proposed, and therefore it remains unclear just how apical EGF stimulation alters the expression of the TJ molecules ZO-1 and occludin. In this regard, although application of EGF is certainly clinically relevant, other members of this family (eg, heparin binding-EGF) can also enhance wound repair, and therefore it would seem important to determine their effects on TJ formation.

Some of the findings of Xiao et al¹³ contrast with recently published data. For example, both Hackett et al² and Parker et al¹⁵ saw no difference in TER between asthmatic and nonasthmatic air-liquid interface cultures from either adults or children. However, it is important to note that the effect of the relatively small changes in TER measured by Xiao et al¹³ was reflected by greater changes in macromolecule permeability. Thus studying changes in paracellular macromolecular transport might provide more clinically relevant data compared with TER.

Are the findings reported by Xiao et al¹³ linked to the cause of asthma? Certainly, it is clear that the expression of E-cadherin, ZO-1, and now occludin-1 in the epithelium is disrupted in asthma. This is seen *in vivo* and is independent of medication use. Strikingly, this phenotype for the most part is maintained *in vitro*, lending significant weight to the developing paradigm that the epithelium is structurally and functionally abnormal in asthmatic patients. However, despite this new information, we are no closer to understanding how or why expression of these proteins is deranged and whether this is a cause or effect of the disease process. Although single nucleotide polymorphisms in E-cadherin have been associated with decreased protein expression,¹⁶ association of junctional proteins with asthma have not been identified in larger genome-wide association studies. The role of epigenetic modifications might therefore be a more plausible reason for the modified expression of cell-cell adhesion

proteins in asthmatic patients, especially given the location of the epithelium at the interface with the inhaled environment. As mentioned above, there are several classes of adhesion complexes, each constructed by several molecules, and thus it is now necessary to determine whether all or some of them are associated with asthma and, if so, how this occurs. For now, the positive effect of (topical) EGF on epithelial integrity and barrier function offers some hope that if the defect or defects in TJs are found to contribute to asthma, then a novel potential treatment option will have been identified.

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