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Targeting HDAC's to restore epithelial barrier integrity: a new option for personalized medicine in allergic airway disorders?

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Airway epithelial cells are an important component of the mucosal immune system, and are key sensors of inhaled allergens, pollutants, and respiratory viruses. In addition to their role as immune sentinels, epithelial cells also form a physical barrier to the outside world. In recent years, epithelial barrier dysfunction has been associated with allergic airway diseases including asthma and allergic rhinitis. Epithelial barrier dysfunction in this context refers to defective expression or function of junction proteins that normally keep cells joined tightly together (1). Defining the mechanisms and consequences of epithelial barrier dysfunction in allergic diseases is an active area of research. In this issue of the *Journal*, Steelant and colleagues describe a new pathway of epithelial barrier dysfunction associated with histone deacetylase (HDAC) activation (2) (see Figure 1). Using an HDAC inhibitor currently in clinical development to treat cancer, Steelant et al. found that it not only enhanced barrier function of epithelial cells in vitro, but also attenuated barrier dysfunction, airway inflammation and hyper-reactivity in a mouse model of house dust mite induced asthma. Taken together with other recent studies, this new report suggests that HDAC inhibitors might find a future home in asthma and allergy clinics.

In 2011, Xiao and Davies reported in this *Journal* that the expression of the tight junction adaptor protein zonula occludens 1 (ZO1) was reduced in bronchial epithelial cells from patients with asthma (3). Steelant and colleagues recently reported a very similar phenotype using nasal epithelial cells obtained from subjects with allergic rhinitis (4). In that report, both ZO-1 and occludin expression were reduced in epithelial cells from subjects with rhinitis, compared to healthy controls. Occludin is a tight junction protein that binds neighboring cells together, and is linked to the actin cytoskeleton and intracellular signaling molecules via adaptor proteins including ZO-1. Therefore, reduced expression of occludin and ZO-1 has the potential to significantly perturb both epithelial barrier function as well as cell signaling.

What are the molecular mechanisms of reduced junctional protein expression in asthma and allergic rhinitis? There is currently no consensus in this area, and more than one mechanism is likely involved. Xiao et al. reported that ZO-1 mRNA expression was *not* reduced in bronchial epithelial cells from asthmatic subjects, and concluded that reduced ZO-1 protein expression occurred at a post-transcriptional level (3). Post-transcriptional downregulation of junctional protein expression has been frequently observed in epithelial cells infected with respiratory viruses, or exposed to inflammatory mediators, and is thought to involve actin remodeling and junction internalization (1). In contrast, Steelant et al. found that ZO-1 and occludin protein and mRNA expression were both reduced in nasal epithelial cells from subjects with allergic rhinitis (4). Since

this occurred in epithelial cells cultured in vitro for several weeks, one possibility is that junctional complex genes are repressed by epigenetic silencing in subjects with allergic rhinitis.

One mechanism of epigenetic silencing involves deacetylation of histone tails by HDAC's, which interferes with transcriptional activation of associated genes. Briefly, in eukaryotes, nuclear DNA wraps around an octamer of histone proteins to form a nucleosome. Nucleosomes then interact to form higher order chromatin structures and ultimately a chromosome. Histones undergo post-translational modifications that are thought to "loosen" chromatin structure and facilitate the binding of transcription factors and RNA polymerases, leading to gene transcription. The acetylation of lysine residues in histone tails is strongly associated with active gene transcription, and is facilitated by histone acetyltransferases. Histone deacetylases, in contrast, remove lysine residues from histone tails and are associated with transcriptional inhibition.

Steelant et al. report that HDAC activity is enhanced in nasal epithelial cells from subjects with rhinitis, and that this correlates inversely with epithelial barrier function (as assessed using transepithelial electrical resistance) (2). There are eighteen known HDAC's, organized into four groups, which vary in tissue distribution and subcellular localization. The expression of HDAC5 and HDAC11 mRNA and protein were enhanced in cells from a few subjects, providing a potential explanation for increased HDAC activity. Because the HDAC inhibitor (called JNJ-26481585) enhanced both barrier function as well as junctional protein immunofluorescence in epithelial cells from rhinitis subjects, the authors conclude that junctional protein expression is directly inhibited by HDAC's in the inflamed epithelium, and that blocking HDAC activity can restore junction protein expression, and thus barrier function, back to normal. In a mouse model of house dust mite induced allergic inflammation, the authors also showed that JNJ-26481585 inhibited airway inflammation and airway hyperactivity (2). This was associated with higher expression of epithelial tight junction proteins, and reduced epithelial barrier dysfunction, which was quantified by measuring the "outside/in" translocation of inhaled FITC-dextran into the blood stream.

Interestingly, there is precedence for the idea that HDAC inhibitors promote junctional protein expression in other cell types. In lung cancer cells, Fukuda et al. found that JNJ-26481585 enhanced the expression of the adherens junction protein E-cadherin (5). Reduced E-cadherin expression is a feature of epithelial to mesenchymal transition (EMT), which is a hallmark of malignant transformation. Therefore, reversal of EMT,

and restoration of epithelial cell barrier function, provide one mechanism explaining the potential efficacy of HDAC inhibitors in treating both epithelial tumors as well as epithelial dysfunction in allergic airway inflammation.

Why is HDAC expression increased in epithelial cells from subjects with rhinitis? Previous work from Wawrzyniak et al. provides one potential explanation (6). These authors showed that HDAC expression is increased in bronchial epithelial cells from asthmatic donors (e.g. HDAC1 and 9), and that numerous HDAC's were induced by treatment with IL-4 and/or IL-13, cytokines that are known to cause epithelial barrier dysfunction on their own (see Figure 1). The observation that multiple HDAC's are increased in asthma and allergic rhinitis suggests that broad spectrum HDAC inhibitors will be needed in order to demonstrate therapeutic efficacy.

Although the idea that HDAC inhibitors directly enhance the expression of junctional complex genes in airway epithelial cells is an attractive model, it seems likely that the molecular mechanisms involved are more complicated. First, experiments using genome wide-expression profiling often show that a minority of genes are directly affected by HDAC inhibition, with roughly similar numbers of genes either enhanced or inhibited. Second, lysine acetylation is emerging as an important post-translational modification of many non-histone proteins (7) (8), and HDAC's can deacetylate both nuclear and cytoplasmic proteins. In fact, it has been suggested that these proteins are better called lysine deacetylases ("KDAC's") to reflect their broad substrate specificity (8). In future studies, it will be important to determine if the reduced expression of junctional proteins in epithelial cells from subjects with asthma and rhinitis is associated with changes in chromatin structure at the respective genes. Third, the effects of JNJ-26481585 on epithelial barrier function in vitro were non-monotonic, with less efficacy at higher doses. Although this could reflect differential affinity for different HDAC's, another possibility is that other non-histone substrates were being targeted. Finally, HDAC inhibitors have been shown to inhibit production of type 2 cytokines in innate lymphoid cells and basophils (9, 10), suggesting that they might have broader effects in the context of allergic airway inflammation.

Asthma is a highly heterogeneous disease, and much current research is aimed at identifying personalized therapies for specific subsets of subjects. The studies by Seelant et al. raise the intriguing notion that subjects with asthma or rhinitis and associated epithelial barrier dysfunction could be treated with HDAC inhibitors. Clinical trials of HDAC inhibitors in cancer patients have demonstrated serious potential side

effects including bone marrow suppression, nausea, vomiting and fatigue. One way to minimize off-target effects and systemic toxicity of these compounds would be to administer them topically to the airways via inhalation. It is currently difficult to quantify epithelial barrier function in individual subjects, but it might be possible in the future to use non-invasive tests of “airway leakiness” to identify subjects with defective junctional protein expression. Since IL-4/IL-13 can induce HDAC expression, and cause barrier dysfunction, subjects with leaky airways should also have elevated levels of Type 2 biomarkers.

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