



Mucosal Barrier Defects: What Have We Learned from Atopic Dermatitis, Asthma, and Allergic Rhinitis?

Prestina Smith¹ · Michael Koval^{1,2} · Joshua M. Levy³

© Springer Science+Business Media, LLC, part of Springer Nature 2020

Abstract

Purpose of the Review This review covers recent findings describing roles for tight junction proteins in the mucosal barrier and their dysfunction in atopic diseases.

Recent Findings Mucosal barrier dysfunction is commonly associated with chronic inflammation. Changes in tight junction protein expression and localization can be indicators of disease progression in allergic rhinitis. In addition, alterations in tight junctions may have a role in the initial onset of asthma. Lastly, polymorphisms in tight junction genes can be early predictors for atopic dermatitis risk and severity.

Summary Tight junctions play a vital role in maintenance of the epithelial barrier and are frequently disrupted in chronic inflammatory disease. Future study is warranted to evaluate novel targeted therapeutics that promote barrier function in the treatment of atopic disease.

Keywords Tight junction · Allergic rhinitis · Atopic dermatitis · Asthma · Claudin · Barrier function

Introduction

The mucosal epithelium acts as a physical barrier between the external environment and the underlying body. Within the upper aerodigestive tract, this barrier serves as the first line of defense against allergens and other pro-inflammatory mediators (i.e., pathogens, toxins) that may be introduced via ingestion or inhalation [1, 2]. Essential to its function in host

defense, the epithelial barrier is selectively permeable, allowing the movement of nutrients and small ions across the barrier while preventing the passage of toxins and pathogens. Movement across the epithelial layer is regulated by tight junctions (TJs) that are formed at sites of epithelial intercellular contact. These TJs bring adjacent cells into close proximity, creating a semi-permeable barrier between cells [3]. Each junction is made of protein complexes that interact both within a cell and between cells across the paracellular space [4]. TJs are made up of transmembrane proteins, cytoplasmic scaffolding and adaptor proteins, and cytoskeletal filaments [5].

Barrier Structure: Claudins, Occludin, and Zonula Occludens Proteins

Claudins are a family of more than 20 distinct transmembrane proteins that serve as the major functional unit of tight junctions and mediate epithelial barrier function throughout the upper and lower airways. Claudin expression is regulated throughout the respiratory tree, and claudins concentrate at sites of highest barrier function such as epithelial cells lining the alveoli [6]. In the lower airway, where the role of claudins in epithelial barrier function is well defined, claudins can be classified based

This article is part of the Topical Collection on *Otolaryngic Allergy*

✉ Joshua M. Levy
joshua.levy2@emory.edu

Prestina Smith
prestina.m.smith@emory.edu

Michael Koval
mhkoval@emory.edu

¹ Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Department of Medicine, Emory University, 205 Whitehead Building, 615 Michael Street, Atlanta, GA 30322, USA

² Department of Cell Biology, Emory University, 205 Whitehead Building, 615 Michael Street, Atlanta, GA 30322, USA

³ Department of Otolaryngology- Head and Neck Surgery, Emory University School of Medicine, 550 Peachtree Street NE, Medical Office Tower, Suite 1135, Atlanta, GA 30308, USA

on their ability to promote or restrict paracellular permeability. One group of claudins (e.g., claudin-2, claudin-10, claudin-15) is considered “leaky” by promoting the formation of pores that permit the passage of small molecules across the epithelium, while another group (e.g., claudin-1, claudin-4, claudin-7, and claudin-8) are considered sealing by restricting movement across the barrier [6]. In the human nasal mucosa, claudin-1, claudin-2, claudin-4, claudin-5, claudin-6, claudin-7, claudin-8, claudin-9, claudin-12, claudin-13, and claudin-14 have been detected either in multiple epithelial layers or in cultured primary nasal epithelial cells [7, 8].

Although claudins are a major class of TJ transmembrane proteins, they are accompanied by other integral proteins at the cell surface. Occludin was the first transmembrane TJ protein to be identified; however, it does not directly contribute to the mechanical paracellular barrier. Instead, multiple studies have demonstrated that occludin is an important regulator of epithelial barrier function in multiple tissues, including the airway and the skin [9].

Functional stability is regulated by the zonula occludens (ZO) scaffolding proteins that have known interactions with various claudins and occludin. ZO proteins (ZO-1, ZO-2, ZO-3) largely function to bind to the actin cytoskeleton and help anchor TJs. ZO proteins are also expressed in epithelial cells throughout the airway where they remain largely localized to the cell borders (Fig. 1).

The contribution of TJs to mucosal barrier dysfunction is an emerging topic. TJ function is often impaired by inflammation, with recent findings underscoring the role of TJs and barrier function in chronic inflammatory disease [10]. Here, we review recent studies identifying roles for TJ proteins in atopic disease, including allergic rhinitis (AR), atopic dermatitis (AD), and asthma.

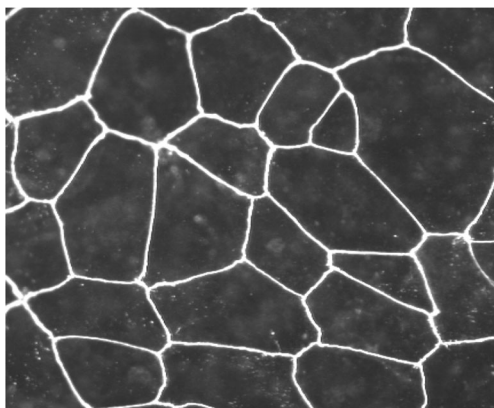


Fig. 1 ZO-1 localization to tight junctions in sinonasal epithelium. Immunostaining of ZO-1 in sinonasal cell monolayer grown 14 days at air-liquid interface. In sinonasal monolayers, ZO-1 localized to tight junctions at apical cell borders (white)

Allergic Rhinitis

AR is characterized by local, type-1 IgE-mediated hypersensitivity reactions to environmental antigens [11]. Mucosal barrier defects are well characterized in AR, often resulting from local inflammation and serving as a route for increased antigen exposure [12]. Recent studies investigating barrier defects in AR have been conducted by Steelant and colleagues using tissue explants from AR patients. These studies used canonical barrier function tests such as transepithelial resistance (TER) and dye flux assays to highlight deficiencies in barrier function associated with AR. In a 2018 study in the *Journal of Allergy and Clinical Immunology*, Steelant et al. found that nasal explants from AR patients showed decreased TER, and displayed an increase in tissue permeability compared with control samples taken from healthy subjects [13]. This study was able to measure barrier dysfunction directly in AR patient samples. Steelant et al. also observed a reduction in both *OCN* (occludin) and *TJP1* (ZO-1) mRNA expression in AR patient samples compared with healthy controls. This reduction was also present at the protein level where a reduction in occludin and ZO-1 protein was measured by immunofluorescence. In contrast, there were no changes in the expression of the barrier promoting claudin-1 and claudin-4 [13]. In addition to providing a mechanistic basis for increased barrier permeability in AR, this study underscores that the barrier forming function of claudins can be modulated by the regulatory functions of decreased occludin and ZO-1 expression without any changes in claudin expression.

Steelant et al. reported similar results in a 2016 study investigating barrier function in house dust mite (HDM)-induced AR tissue biopsies [14]. They found decreased TER, increased dye permeability, and decreased occludin and ZO-1 mRNA and protein levels in HDM-induced AR patient samples compared with healthy controls. This phenotype persisted in cell culture where HDM-induced AR nasal epithelial cells were dissociated from tissue and grown in cultured monolayers. AR nasal epithelial cells displayed the same relative one third reduction in TER, compared with healthy controls, that was observed in the tissue samples and a similar decrease in occludin and ZO-1 mRNA and protein expression. Treating these cells with intranasal corticosteroids, a first-line therapy used to treat AR restored both TER and occludin/ZO-1 expression to baseline. Similarly, tissue samples taken from AR patients who had undergone intranasal steroid treatment showed higher occludin and ZO-1 expression, compared with non-treated AR patients, suggesting that intranasal steroid treatment is able to restore barrier function by regulating TJ expression in HDM-induced AR patients.

Researchers have been able to model initiators of AR in murine and cell culture models using allergens, HDM. Recent in vitro models of AR have used the HDM-associated cysteine protease Der p1 to mimic the allergic response seen in AR

patients. In these studies, epithelial cells were isolated from human sinonasal biopsies, grown to confluency, and cultured at air-liquid interface (ALI) to simulate the microenvironment of the human airway. These cells were then co-treated with Der p1 activated by co-treatment with dithiothreitol (DTT) in order to induce an allergic response. In a 2013 study published in the *International Forum of Allergy and Rhinology*, Henriquez and colleagues used Der p1 + DTT treatment to investigate the function of allergens and barrier function in human sinonasal epithelial cells [15]. They found that Der p1 + DTT decreased TER in human sinonasal cells as compared with mock-treated controls [15]. Furthermore, the study showed that Der p1 + DTT treatment decreased claudin-1 protein levels by immunoblot analysis and diminished claudin-1 localization at the cell borders of sinonasal epithelial cells. Lastly, the study reported no change in ZO-1 and occludin protein levels. This study demonstrated that Der p1 reduces the expression and TJ localization of barrier forming claudin-1, suggesting a pathway that HDM allergens may use to traverse the mucosal barrier contributing to the AR phenotype.

Allergic Asthma

In addition to AR, chronic inflammation has also been implicated as a driver of barrier defects in asthma [16]. Recent studies investigating the role of TJ proteins and barrier function in asthma have used lower airway epithelial samples isolated from patients. In a 2011 study published in the *Journal of Allergy and Clinical Immunology*, Xiao et al. reported culturing monolayers of bronchial epithelial cells obtained from lung brushings from patients with mild, moderate, or severe asthma [17]. After growing at ALI, bronchial epithelial monolayers from patients with moderate or severe asthma had decreased TER as compared with healthy controls. The group also found that monolayers from moderate or severe asthma patients had increased dye permeability compared with healthy controls consistent with a decrease in barrier function. In addition, Xiao and colleagues measured occludin and ZO-1 expression and found a reduction in ZO-1 levels and a trending reduction in occludin in bronchial epithelial cells from patients with asthma [17]. These findings were consistent with a previous study conducted by DeBoer et al. that reported decreased ZO-1 levels in bronchial cells isolated from asthma patients [18].

Alterations in TJ proteins have also been observed in murine models of asthma. In a recent study published in *Allergy*, Tan et al. treated mice with HDM extract to induce allergic airway inflammation [19•]. Transcriptomic analysis of lungs isolated from treated mice identified differential expression of various TJ genes. The group found that *TJPI* (ZO-1) mRNA and protein was reduced and *CLDN18* mRNA was decreased in HDM-treated mice, compared with control. Claudin-18

deficiency was also identified in human airway cells from asthmatic patients. A study conducted by Sweerus and colleagues found that *CLDN18* mRNA is reduced in airway epithelial brush samples from patients with asthma, compared with healthy controls [20•].

The studies conducted in bronchial epithelial cells underscore the importance of TJ proteins in the early stages of allergic asthma. Disruption of the bronchial epithelial barrier can facilitate the passage of allergens into the airway epithelium. This leads to immune activation and local inflammation characteristic of this atopic disease.

Atopic Dermatitis

Much like the bronchial epithelium, the skin is a boundary tissue that creates a barrier to prevent the internal passage of inflammatory mediators. AD is a chronic inflammatory disease associated with disruption of this barrier, affecting an estimated 20% of the population in developed countries [21]. Clinically, AD is characterized by recurrent eczema and chronic pruritis that greatly impacts quality of life. Two structures are mainly responsible for maintaining the epithelial barrier in the skin: the stratum corneum and TJs [22•]. The stratum corneum is the outermost layer of the skin epidermis and consists of piles of dead keratinocytes. Underneath the stratum corneum, the tight junctions form a secondary barrier. Barrier dysfunction in AD has been identified and mainly attributed to mutations in filaggrin, a filament aggregating protein that stabilizes keratin fibers in keratinocyte cytoskeleton and contributes to the overall strength of the skin [23]. Current studies, however, have begun to uncover the role of TJs in AD.

In a 2011 study published in the *Journal of Allergy and Clinical Immunology*, de Benedetto and colleagues investigated barrier function and the role of claudin-1 in AD. The group reported decreased TER in isolated epidermis from AD patients compared with non-atopic control samples [24]. In addition, the group found an increase in the permeability of fluorescently labeled albumin across the AD epidermis compared with controls, consistent with a decrease in barrier function. Next, de Benedetto et al. performed gene expression profiling in blister roofs of patients with AD, limiting their assay to proteins known to be important for barrier function. The group reported that claudin-1 and claudin-23 expression was decreased in AD patients compared with controls [24]. Similarly, claudin-1 protein levels were decreased in AD epidermis compared with non-atopic samples as measured by immunofluorescence. Finally, the group tested whether *CLDN1* gene variants are associated with a risk for AD by genotyping patients with AD and found 2 single nucleotide polymorphism (SNPs), 1 non-coding and 1 in the *CLDN1* promoter, that were associated with greater AD severity in two independent North American populations. These findings

have since been corroborated in other populations. In a 2016 study, Asad et al. assayed *CLDN1* SNPs from Ethiopian patients with AD and found an association between rs893051, a *CLDN1* SNP, and AD [25••]. These studies indicated that *CLDN1* may be involved in the susceptibility of AD in multiple groups.

Another study published in 2016 by Yuki et al. identified other TJ proteins altered in AD. This group analyzed non-lesion versus lesion sites from AD skin biopsies by immunohistochemistry and western blotting for TJ proteins [26•]. Yuki et al. reported that ZO-1 protein levels were reduced in non-lesion sites from AD skin biopsies compared with control samples. In contrast, ZO-1 and claudin-1 levels were decreased in the lesion sites of AD patient biopsies compared with control. The group reported no change in occludin.

These studies conducted in tissue samples biopsied from AD patients have established TJ proteins as indicators for disease. Claudin expression is not only altered in AD but was also shown to be an indicator for disease susceptibility and severity. ZO-1 protein levels were reduced in AD, even in areas without lesions, suggesting a role for TJ proteins as early predictors of AD.

Clinical Applications

Targets to maintain epithelial barrier function represent novel approaches for the treatment of allergic airway inflammation. Recent translational studies have evaluated targeted therapies to stabilize the disrupted barrier, with encouraging preliminary findings. Steelant et al. recently described histone deacetylase (HDAC) as a novel mediator of epithelial barrier dysfunction. Interestingly, they repurposed a broad spectrum HDAC inhibitor, JNJ-26481585, currently in trials for the treatment of cancer [27••]. Steelant et al. found that treating sinonasal cells from patients with AR with concentrations of JNJ-26481585 as low as 0.5 nM could significantly increase TER values compared with mock-treated AR samples. In addition, treatment increased ZO-1 and occludin expression. This effect was not limited to in vitro experiments. In an in vivo murine model of HDM-induced allergic airway inflammation, JNJ-26481585 restores airway expression of ZO-1 and occludin, leading to improvement in pathologic nasal epithelial permeability. These preliminary findings give insight into future novel therapeutic studies aiming to decrease atopic inflammation and associated patient symptoms via restoration of the epithelial barrier.

Conclusions

While AR, asthma, and AD all share inflammation and barrier dysfunction as common features of their pathology, they differ in the mechanism underlying this disruption. One

consequence is that a “one size fits all” approach to promote barrier function in these diseases will not be efficacious and instead the context in which TJs are disrupted needs to be considered. Nonetheless, identifying markers for susceptibility to barrier impairment in specific diseases and screening of pharmacologic agents for the ability to re-normalize TJ protein expression show great promise. The ability to restore proper epithelial barrier function in AR, asthma, and AD is likely to provide a powerful adjunctive therapeutic approach in the treatment of these and other diseases attributable to inflammation and allergy.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent All procedures performed in studies involving human participants were in accordance with the ethical standards of the Emory University Institutional Review Board (IRB00102406) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. France MM, Turner JR. The mucosal barrier at a glance. *J Cell Sci*. 2017;130(2):307–14. <https://doi.org/10.1242/jcs.193482>.
2. Schleimer RP, Kato A, Kern R, Kuperman D, Avila PC. Epithelium: at the interface of innate and adaptive immune responses. *J Allergy Clin Immunol*. 2007;120(6):1279–84. <https://doi.org/10.1016/j.jaci.2007.08.046>.
3. Zihni CM, C, Matter K, Balda M. Tight junctions: from simple barriers to multifunctional molecular gates. *Nat Rev Mol Cell Biol*. 2016;17. <https://doi.org/10.1038/nrm.2016.80>.
4. Van Itallie CMAJ. Architecture of tight junctions and principles of molecular composition. *Semin Cell Dev Biol*. 2014;36:157–65. <https://doi.org/10.1016/j.semcdb.2014.08.011>.
5. Citi S. The mechanobiology of tight junctions. *Biophys Rev*. 2019;11(5):783–93. <https://doi.org/10.1007/s12551-019-00582-7>.
6. Schlingmann B, Molina SA, Koval M. Claudins: gatekeepers of lung epithelial function. *Semin Cell Dev Biol*. 2015;42:47–57. <https://doi.org/10.1016/j.semcdb.2015.04.009>.
7. Kojima T, Go M, Takano K, Kurose M, Ohkuni T, Koizumi J, et al. Regulation of tight junctions in upper airway epithelium. *Biomed Res Int*. 2013;2013:947072. <https://doi.org/10.1155/2013/947072>.
8. Fukuoka A, Yoshimoto T. Barrier dysfunction in the nasal allergy. *Allergol Int*. 2018;67(1):18–23. <https://doi.org/10.1016/j.alit.2017.10.006>. **A review that describes the relationship between TJ disruption and allergic diseases.**
9. Feldman GJ, Mullin JM, Ryan MP. Occludin: structure, function and regulation. *Adv Drug Deliv Rev*. 2005;57(6):883–917. <https://doi.org/10.1016/j.addr.2005.01.009>.

- 10.● Steelant B, Seys SF, Boeckstaens G, Akdis CA, Ceuppens JL, Hellings PW. Restoring airway epithelial barrier dysfunction: a new therapeutic challenge in allergic airway disease. *Rhinology*. 2016;54(3):195–205. <https://doi.org/10.4193/Rhin15.376>. **A review highlighting the link between epithelial barrier dysfunction and allergic diseases. In addition to airway diseases, this review describes the role of TJs in other chronic inflammatory conditions such as AD, Chron's disease and ulcerative colitis.**
- 11.● Wise SK, Lin SY, Toskala E, Orlandi RR, Akdis CA, Alt JA, et al. International consensus statement on allergy and rhinology: allergic rhinitis. *Int Forum Allergy Rhinol*. 2018;8(2):108–352. <https://doi.org/10.1002/alf.22073>. **Comprehensive review of allergic rhinitis including pathophysiology and patient management.**
12. London NR Jr, Ramanathan M Jr. The role of the sinonasal epithelium in allergic rhinitis. *Otolaryngol Clin N Am*. 2017;50(6):1043–50. <https://doi.org/10.1016/j.otc.2017.08.002>.
- 13.● Steelant B, Seys SF, Van Gerven L, Van Woensel M, Farre R, Wawrzyniak P, et al. Histamine and T helper cytokine-driven epithelial barrier dysfunction in allergic rhinitis. *J Allergy Clin Immunol*. 2018;141(3):951–63 e8. <https://doi.org/10.1016/j.jaci.2017.08.039>. **This study identifies allergic inflammatory mediators driving epithelial barrier dysfunction in AR. In addition, this study describes the epithelial barrier and TJ proteins in AR patient samples.**
- 14.● Steelant B, Farre R, Wawrzyniak P, Belmans J, Dekimpe E, Vanheel H, et al. Impaired barrier function in patients with house dust mite-induced allergic rhinitis is accompanied by decreased occludin and zonula occludens-1 expression. *J Allergy Clin Immunol*. 2016;137(4):1043–53 e5. <https://doi.org/10.1016/j.jaci.2015.10.050>. **This study describes nasal epithelial barrier dysfunction and TJ protein disruption in HDM-induced AR patient samples.**
15. Henriquez OA, Den Beste K, Hoddeson EK, Parkos CA, Nusrat A, Wise SK. House dust mite allergen Der p 1 effects on sinonasal epithelial tight junctions. *Int Forum Allergy Rhinol*. 2013;3(8):630–5. <https://doi.org/10.1002/alf.21168>.
16. Gon Y, Hashimoto S. Role of airway epithelial barrier dysfunction in pathogenesis of asthma. *Allergol Int*. 2018;67(1):12–7. <https://doi.org/10.1016/j.alit.2017.08.011>.
17. Xiao C, Puddicombe SM, Field S, Haywood J, Broughton-Head V, Puxeddu I, et al. Defective epithelial barrier function in asthma. *J Allergy Clin Immunol*. 2011;128(3):549–56 e1–12. <https://doi.org/10.1016/j.jaci.2011.05.038>.
18. de Boer WI, Sharma HS, Baelemans SM, Hoogsteden HC, Lambrecht BN, Braunstahl GJ. Altered expression of epithelial junctional proteins in atopic asthma: possible role in inflammation. *Can J Physiol Pharmacol*. 2008;86(3):105–12. <https://doi.org/10.1139/y08-004>.
- 19.● Tan HT, Hagner S, Ruchti F, Radzikowska U, Tan G, Altunbulakli C, et al. Tight junction, mucin, and inflammasome-related molecules are differentially expressed in eosinophilic, mixed, and neutrophilic experimental asthma in mice. *Allergy*. 2019;74(2):294–307. <https://doi.org/10.1111/all.13619>. **This study describes the differential expression of TJ proteins and inflammatory mediators in multiple murine models of house dust mite-induced asthma.**
- 20.● Sweerus K, Lachowicz-Scroggins M, Gordon E, LaFemina M, Huang X, Parikh M, et al. Claudin-18 deficiency is associated with airway epithelial barrier dysfunction and asthma. *J Allergy Clin Immunol*. 2017;139(1):72–81. <https://doi.org/10.1016/j.jaci.2016.02.035>. **e1 This study identifies decreased claudin-18 levels in human airway cells from patients with asthma. In addition, loss of claudin-18 led to barrier dysfunction in 16HBE cells and in mice.**
21. Rerknimitr P, Otsuka A, Nakashima C, Kabashima K. The etiopathogenesis of atopic dermatitis: barrier disruption, immunological derangement, and pruritus. *Inflamm Regen*. 2017;37:14. <https://doi.org/10.1186/s41232-017-0044-7>.
- 22.● Egawa G. Pathomechanism of 'skin-originated' allergic diseases. *Immunol Med*. 2018;41(4):170–6. <https://doi.org/10.1080/25785826.2018.1540257>. **This review describes the structure of the skin barrier and the role of TJs in the skin. In addition, the review covers recent findings implicating claudin-1 in AD.**
23. Agrawal R, Woodfolk JA. Skin barrier defects in atopic dermatitis. *Curr Allergy Asthma Rep*. 2014;14(5):433. <https://doi.org/10.1007/s11882-014-0433-9>.
24. De Benedetto A, Rafaels NM, McGirt LY, Ivanov AI, Georas SN, Cheadle C, et al. Tight junction defects in patients with atopic dermatitis. *J Allergy Clin Immunol*. 2011;127(3):773–86 e1–7. <https://doi.org/10.1016/j.jaci.2010.10.018>.
- 25.● Asad S, Winge MC, Wahlgren CF, Bilcha KD, Nordenskjold M, Taylan F, et al. The tight junction gene Claudin-1 is associated with atopic dermatitis among Ethiopians. *J Eur Acad Dermatol Venereol*. 2016;30(11):1939–41. <https://doi.org/10.1111/jdv.13806>. **This study characterizes CLDN1 polymorphisms as indicators of AD susceptibility in the Ethiopian population.**
- 26.● Yuki T, Tobiishi M, Kusaka-Kikushima A, Ota Y, Tokura Y. Impaired tight junctions in atopic dermatitis skin and in a skin-equivalent model treated with interleukin-17. *PLoS One*. 2016;11(9):e0161759. <https://doi.org/10.1371/journal.pone.0161759>. **This study finds TJ alterations and barrier dysfunction in skin biopsies from patients with AD.**
- 27.● Steelant B, Wawrzyniak P, Martens K, Jonckheere AC, Pugin B, Schrijvers R, et al. Blocking histone deacetylase activity as a novel target for epithelial barrier defects in patients with allergic rhinitis. *J Allergy Clin Immunol*. 2019. <https://doi.org/10.1016/j.jaci.2019.04.027>. **This study identifies increased HDAC activity as a mechanism contributing to epithelial barrier dysfunction. This study also identifies a HDAC inhibitor as a promising novel therapeutic for inflammatory airway diseases.**

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.