SPLUNC1 α6 Peptidomimetic: A Novel Therapeutic for Asthma

Asthma is a disease characterized by chronic airway inflammation and reversible airflow limitation. This inflammation involves activated mast cells, infiltration of eosinophils, neutrophilia, mucus accumulation, and increases in the number of activated T-helper cell type 2 cells, which orchestrate and perpetuate allergic inflammation through the release of various cytokines and chemokines (1, 2). People with severe asthma experience persistent symptoms despite the use of conventional medications including long-acting β_2-adrenergic receptor agonists, muscarinic antagonists, leukotriene receptor antagonists, and inhaled corticosteroids for reasons that are not fully understood (1, 2). Therefore, there is a clinical and urgent need for new therapies to effectively treat people with severe asthma.

It is well recognized that many of the pathogenic processes in asthmatic airways are dependent on increases in cytoplasmic Ca^{2+} and include events such as excitation–contraction coupling in smooth muscle, stimulus–secretion coupling in mast cells and mucous glands, nerve impulse initiation and conduction, and inflammatory cell infiltration (2, 3). Orai1 is a plasma membrane Ca^{2+} channel that is involved in store-operated Ca^{2+} entry, and Orai1/store-operated Ca^{2+} entry are upstream of transcription factors that promote inflammation (4–6). It is therefore conceivable that inhibition of Orai1 could be a novel way of reducing inflammation in a range of conditions including asthma. Wu and colleagues have previously shown that the multifunctional protein SPLUNC1 (Short Palate LUng and Nasal epithelial Clone 1), via its sixth α-helical (α6) region, regulates Orai1 and Ca^{2+} signaling (7). Moreover, studies have shown that SPLUNC1 is expressed in airway epithelia and secreted into the lung lumen where it regulates processes such as smooth muscle contraction and inflammation (7–10). Thus, there is a case for the development of peptidomimetics of SPLUNC1's α6 region to inhibit Orai1 to reduce pulmonary inflammation.

In this issue of the *Journal*, Wrennall and colleagues (pp. 271–282) report on their examination of the therapeutic potential of a novel α6 peptidomimetic for asthma (11). They did this by first investigating the role of SPLUNC1 in allergy-induced asthma by exposing wild-type (WT) and SPLUNC1$^{-/-}$ mice to house dust mite (HDM) allergen. It was interesting to note that HDM exposure resulted in a significantly greater increase in eosinophil counts in SPLUNC1$^{-/-}$ mice than in WT littermates, suggesting a hyperinflammatory response. These findings are consistent with those of Thaikoottathil and colleagues showing that SPLUNC1 deficiency enhances airway eosinophilic inflammation in mice (9), indicating that SPLUNC1 may play a role in modulating the T-helper cell type 2 immune response in the lung. The concept of SPLUNC1 playing a role in asthma is further supported by studies showing that SPLUNC1 is a gene modifier for asthma (12) and that SPLUNC1 levels are reduced in people with asthma (7).

Having established a role for SPLUNC1 in allergic inflammation, the authors then proceeded to generate a peptide based on SPLUNC1's α6 region to test whether it could reduce Orai1 levels in HDM-exposed mice after intranasal delivery. BAL immune cells from SPLUNC1$^{-/-}$ mice treated with HDM had significantly greater Orai1 expression than BAL cells obtained from WT littermate control mice. Interestingly, intranasally delivered α6 reduced Orai1 expression in BAL immune cells obtained from both WT and SPLUNC1$^{-/-}$ mice, suggesting that Orai1 expression in BAL immune cells may play a role in the sensitization of inflammatory responses to allergens and that this effect may be regulated by SPLUNC1. Given that SPLUNC1$^{-/-}$ mice displayed exaggerated inflammatory responses to HDM exposure and that α6 reduced Orai1 expression in BAL immune cells, it was no surprise that the α6 peptide inhibited airway inflammation in HDM-exposed mice.

The therapeutic potential of aerosolized therapies for asthma is dependent on several factors including the particle size of the drug, the dose deposited, its distribution within the lung, and whether pathological features of the disease impede drug delivery via inhalation. Given that it is well recognized that mucus can interact with proteins and peptides, the authors accordingly sought to determine whether their formulated α6 peptide interacts with mucus, which would impede delivery via inhalation. Quartz crystal microbalance with dissipation analysis demonstrated that α6 does not interact with mucus. Moreover, scanning EM revealed that jet milled α6 displayed a consistent particle size that was suitable for inhalation to the lower airways. Collectively, the data indicate that the α6 peptide is a viable candidate for dry powder inhalation and importantly that its activity was not affected by jet milling or nebulization.

There are potential limitations to this study that bear some discussion. First, the authors have shown that α6 neither interacts with nor penetrates the epithelial barrier, suggesting that α6 imparts its antiinflammatory effects solely through interactions with immune cells already recruited to the airway lumen. The authors propose that this could potentially be through α6 reducing Ca^{2+}-dependent cytokine release from these cells and/or reducing the residence time of eosinophils in the airway lumen. Further mechanistic studies to fully understand the extent of α6 penetration into the lung, and its target cell types, are required. Second, it is well recognized that people with severe asthma have significant airway wall remodeling. Although the authors show antiinflammatory effects of the α6 peptidomimetic, it would have been worth investigating whether it can also reduce airway wall remodeling observed in severe asthma. In saying this, the authors have shown in other studies that the α6-related peptides reduce histological signs of airway damage in murine models of bacterial pneumonia, suggesting that α6 may provide protection against airway remodeling. Finally, the authors show that the α6 peptidomimetic reduces lung inflammation, but it would have been worth investigating whether this reduced lung inflammation translated to improvements in lung function. Despite these potential limitations, this valuable study will inform and prompt further studies in the field of asthma that will expand on the outcomes of the present study.

Originally Published in Press as DOI: 10.1165/rcmb.2021-0451ED on December 14, 2021

In summary, this study by Wrennall and colleagues has shown that administration of a SPLUNC1 α6 peptidomimetic reduces HDM-induced airway inflammation in mice and that the α6 peptide may serve as a novel therapeutic for the treatment of asthma where conventional treatments do not work or where nonsteroidal alternatives are needed. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Ross Vlahos, Ph.D.
School of Health and Biomedical Sciences
RMIT University
Bundoora, Victoria, Australia

ORCID ID: 0000-0001-8443-7071 (R.V.).

References

1. Holgate ST, Wenzel S, Postma DS, Weiss ST, Renz H, Sly PD. Asthma. *Nat Rev Dis Primers* 2015;1:15025.
2. Barnes PJ. Cellular and molecular mechanisms of asthma and COPD. *Clin Sci (Lond)* 2017;131:1541–1558.
3. Mahn K, Ojo OO, Chadwick G, Aaronson PI, Ward JP, Lee TH. Ca(2+) homeostasis and structural and functional remodelling of airway smooth muscle in asthma. *Thorax* 2010;65:547–552.
4. Smyth JT, Hwang SY, Tomita T, DeHaven WI, Mercer JC, Putney JW. Activation and regulation of store-operated calcium entry. *J Cell Mol Med* 2010;14:2337–2349.
5. Lee KP, Yuan JP, Hong JH, So I, Worley PF, Muallem S. An endoplasmic reticulum/plasma membrane junction: STIM1/Orai1/TRPCs. *FEBS Lett* 2010;584:2022–2027.
6. Deng X, Wang Y, Zhou Y, Soboloff J, Gill DL. STIM and Orai: dynamic intermembrane coupling to control cellular calcium signals. *J Biol Chem* 2009;284:22501–22505.
7. Wu T, Huang J, Moore PJ, Little MS, Walton WG, Fellner RC, *et al.* Identification of BPIFA1/SPLUNC1 as an epithelium-derived smooth muscle relaxing factor. *Nat Commun* 2017;8:14118.
8. Di YP. Functional roles of SPLUNC1 in the innate immune response against Gram-negative bacteria. *Biochem Soc Trans* 2011;39:1051–1055.
9. Thaikoottathil JV, Martin RJ, Di PY, Minor M, Case S, Zhang B, *et al.* SPLUNC1 deficiency enhances airway eosinophilic inflammation in mice. *Am J Respir Cell Mol Biol* 2012;47:253–260.
10. Ou C, Sun Z, Zhang H, Xiong W, Ma J, Zhou M, *et al.* SPLUNC1 reduces the inflammatory response of nasopharyngeal carcinoma cells infected with the EB virus by inhibiting the TLR9/NF-κB pathway. *Oncol Rep* 2015;33:2779–2788.
11. Wrennall JA, Ahmad S, Worthington EN, Wu T, Gorlounova AS, Voeller AS, *et al.* A SPLUNC1 peptidomimetic inhibits Orai1 and reduces inflammation in a murine allergic asthma model. *Am J Respir Cell Mol Biol* 2022;66:271–282.
12. Schaefer N, Li X, Seibold MA, Jarjour NN, Denlinger LC, Castro M, *et al.* The effect of BPIFA1/SPLUNC1 genetic variation on its expression and function in asthmatic airway epithelium. *JCI Insight* 2019;4:e127237.