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INSIGHTS

TRPA1: An asthma target with a zing

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Asthma therapy has advanced remarkably; however, a significant number of patients respond poorly to current interventions. Balestrini et al. (2021. *J. Exp. Med.* https://doi.org/10.1084/jem.20201637) advance the concept that sensory nerves control inflammation in asthma, demonstrating that a novel inhibitor of TRPA1, a nerve receptor for irritants and reactive endogenous mediators, suppresses inflammation and airway smooth muscle contraction in several preclinical species.

The introduction of biologics has improved asthma control in a significant number of patients (Pavord et al., 2018). Biologics that target cytokine signaling (IL-5, IL-5R, IL-13, IL4Rα, or thymic stromal lymphopoietin) suppress inflammation and exacerbations in patients affected by Type-2 asthma, driven by Th2 lymphocytes that elicit eosinophilia, inflammation, airway remodeling and bronchoconstriction leading to wheezing (Pavord et al., 2018). The discovery of Type-2–specific biomarkers such as periostin, exhaled nitric oxide (FeNO), and blood eosinophil counts enabled the categorization of patient populations for treatment optimization (Pavord et al., 2018). Together with the use of short- and long-acting agonists and inhaled corticosteroids, mortality from asthma has been substantially reduced and quality of life improved (Pavord et al., 2018).

However, a significant number of asthma patients remain who respond poorly to current therapeutic regimens. These include patients with Type-1 or mixed Type-1/2 asthma in which neutrophils contribute to inflammation. Biomarkers predicting exacerbations remain elusive, making it difficult to identify patients progressing to severe asthma. These shortcomings suggest that additional pathological mechanisms contribute to the etiology of asthma (Pavord et al., 2018).

Sensory nerves in inflammation control in asthma

In this issue, Balestrini et al. demonstrate that selective inhibition of TRPA1 (transient receptor potential [TRP] cation channel member A1), an irritant-activated ion channel in lung-innervating sensory nerves, diminishes pulmonary inflammation and airway hyperreactivity in asthma models in mice and guinea pigs (Balestrini et al., 2021). TRPA1, initially discovered as the target for allyl isothiocyanate (AITC, mustard oil), the pungent ingredient in mustard and wasabi, is a sensory nerve receptor for a wide range of chemical irritants (Bautista et al., 2006; Jordt et al., 2004). These include aldehydes in smoke, tear gas agents, oxidants such as chlorine gas, and particulates (Andrè et al., 2008; Bautista et al., 2006; Bessac et al., 2009; Bessac et al., 2008). The sensory nerves innervating the lower airways and lungs originate in the jugular and nodose ganglia of the vagus nerve. They monitor changes in the chemical environment and respond to physical stimuli such as mechanical stress or changes in temperature. Activation of vagal nerve endings by chemical irritants, particulates, or mucus buildup triggers the cough reflex, bronchoconstriction, and secretions from submucosal glands. Upon activation, sensory nerves release neuropeptides such as substance P and calcitonin gene–related peptide (CGRP) that elicit plasma extravasation,

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swelling, and edema, and activate cellular inflammatory pathways in resident tissue and immune cells, a process named neurogenic inflammation. Chemical or surgical ablation of sensory nerves was shown to reduce asthmatic inflammation in animal models. In the 1990s, inhibitors of the receptor for substance P, neurokinin 1 receptor, diminished inflammation in animal models of asthma; however, clinical trials in asthmatics failed to demonstrate benefits (Boot et al., 2007).

TRPA1 belongs to a group of ion channels that serve as primary sensors for chemical and physical stimuli in sensory neurons. These include additional thermo- and chemosensitive TRP ion channels, purinergic

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receptors (P_2X) and PIEZO mechanosensors. In addition to external stimuli, TRPA1 is activated by endogenous reactive oxidant species and oxidized lipids generated in inflammatory states. Inhibitors of TRPA1 were initially developed for the treatment of pain, since TRPA1 is also expressed in the pain-signaling neurons of the dorsal root and trigeminal ganglia. However, while inhibition of TRPA1 diminished acute chemically induced pain, these inhibitors had little or contradictory effects in animal models of chronic pain.

Studies using TRPA1 inhibitors in models of respiratory pathologies showed more promise. A first-generation inhibitor of TRPA1 diminished airway inflammation and hyperreactivity in mouse model and guinea pig models of allergic asthma, using egg white albumin (OVA) as an allergen (Caceres et al., 2009; Raemdonck et al., 2012). TRPA1 inhibitors also reduced cough and improved survival in animal models of chemical inhalation exposure. These effects were recapitulated in TRPA1-deficient mice and rats (Caceres et al., 2009; Reese et al., 2020).

An advanced TRPA1 inhibitor reduces asthmatic pulmonary inflammation

Early TRPA1 inhibitors lacked the selectivity, potency, and favorable drug-like properties required to be advanced in clinical studies. Balestrini et al. report anti-asthmatic effects of an advanced TRPA1 inhibitor, GDC-0334, with dramatically improved potency (1.7 nM on human TRPA1) and favorable pharmacokinetics in preclinical species (Balestrini et al., 2021). Using cryogenic electron microscopy, Balestrini et al. resolve the structure of purified TRPA1 channels in complex with GDC-0334 at 3.6 Å resolution, revealing that the inhibitor binds near the pore-forming region where it constrains pore opening. GDC-0334, given orally, inhibited chemically induced vascular leakage in rats and reduced irritant-induced dermal blood flow in rats and guinea pigs, a typical neurogenic inflammatory response. In a human clinical trial, orally dosed GDC-0334 prevented the increase in dermal blood upon skin application of AITC as a TRPA1-activating stimulus, and reduced pain and itch sensations.

When testing GDC-0334 in the OVA-induced asthma model in rats, with the inhibitor administered orally before airway challenge with the allergen, Balestrini et al. observed a significant reduction in eosinophil and neutrophil counts in the bronchoalveolar lavage fluid, as well as a reduction in substance P levels. In guinea pigs, GDC-0334 also reduced lung-infiltrating cells and blocked cough triggered by irritant inhalation. The efficacy of GDC-0334 was similar to dexamethasone.

Neuronal TRPA1 drives asthmatic inflammation in mice

TRPA1 was initially found to be exclusively expressed in peripheral sensory neurons; however, more recent studies suggest a more broad non-neuronal distribution of TRPA1, including in the lung (Jordt et al., 2004; Nassini et al., 2012). Comparing expression in mice, rats, guinea pigs, and humans, Balestrini et al. detected TRPA1 in sensory nerves of all species and, at much lower levels, in pulmonary smooth muscle cells and fibroblasts isolated from guinea pigs and humans, but not from mice. Using a diphtheria toxin–based strategy for timed selective cell ablation in mice, the authors eliminated TRPA1-expressing sensory nerves 3 wk after allergen sensitization, resulting in clearly diminished inflammatory responses after subsequent respiratory allergen challenge. A neuronal cell–specific knockout of the TRPA1 gene also resulted in diminished inflammation cell infiltration and IL-5 cytokine expression, as observed in in the global TRPA1 knockout strain (Caceres et al., 2009). These findings strongly suggest that neuronal TRPA1 is the major driver of asthmatic inflammation in mice.

Potential contributions of nonneuronal TRPA1 in other species

Balestrini et al. and others detected expression of TRPA1 mRNA in cultured guinea pig and human pulmonary smooth muscle cells and fibroblasts. Superfusion of smooth muscle cells with GDC-0334 prohibited AITC-induced calcium influx, suggesting presence of functional TRPA1 channels in the cell membrane. GDC-0334 also inhibited the contraction of cultured human smooth muscle cells.

Does TRPA1 expressed in airway smooth muscle cells trigger bronchoconstriction in vivo? In mice, treatment with a TRPA1 inhibitor, or deletion of the TRPA1 gene, dramatically reduced pulmonary resistance in response to contractile stimuli in the OVA asthma model (Caceres et al., 2009). Since mice do not express TRPA1 in airway smooth muscle cells, neuronal TRPA1 appears to be sufficient for establishing asthma-induced pulmonary resistance. It remains unclear whether TRPA1 expression levels in cultured human or guinea pig smooth muscle cells resembles levels in native tissue in vivo. Previously, it was reported that TRPA1 mRNA levels are rapidly up-regulated in human pulmonary fibroblasts and other cell types after tissue dissociation, while only minimal levels are detected in native human tissue (Jaquemar et al., 1999). Thus, the contribution of nonneuronal TRPA1 in smooth muscle contraction in vivo remains to be clarified.

TRPA1 in asthma control: A link between asthma and environmental exposures?

The development of an orally available selective and potent TRPA1 inhibitor with anti-asthmatic activity in animal models and safety and efficacy in humans represents a significant advance, enabling future clinical studies. Whether inhibition of human TRPA1 is an effective anti-asthmatic strategy remains to be established. The OVA model of asthma used by Balestrini et al. has significant limitations. It is an acute transient inflammation model, while human asthma is a chronic disease associated with tissue remodeling and highly divergent pathologies. Chronic asthma models may reveal whether TRPA1 inhibition is an effective strategy for treatment of human asthma.

How does TRPA1 control inflammation and airway hyperreactivity in the OVA model? OVA sensitization and challenge elicit a Type-2 asthmatic response. Blood levels of OVA-reactive IgE are indistinguishable in OVA-sensitized wild-type and TRPA1-deficient mice, suggesting that TRPA1 is redundant for establishing the systemic immune response to allergens (Caceres et al., 2009). However, TRPA1-deficient mice clearly fail to mobilize the immune response to the site of allergen challenge. Neurogenic inflammation as a directional cue remains a favorite hypothesis. TRPA1 does not only control substance P release from sensory nerve endings, but also the release of CGRP and other neuropeptides and transmitters interacting with local tissue cells and cells of the immune system, acting as a gatekeeper of neurogenic inflammation (Bautista et al., 2013). Pro-asthmatic neuropeptides are also released

from pulmonary neuroendocrine cells (Sui et al., 2018). Neuroendocrine cells in other epithelial organs are known to connect to underlying sensory nerves and express TRPA1, and such interactions should be investigated in the lung (Ye et al., 2021). Other mechanisms may involve the control of pro- and anti-inflammatory sensory-autonomic reflex circuits. TRPA1, as a target for environmental chemical exposures, is likely to promote exposure-associated asthma exacerbations that frequently occur during pollution events associated with increased ozone levels, smog, and wildfires that also increase cough frequencies in certain subpopulations of asthmatics. Heightened TRPA1-mediated chemical sensitivity should be investigated as a biomarker predicting asthma exacerbations and transition to severe asthma.

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