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Trpa1 In Airway Inflammation

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REGULATION OF TRP ION CHANNELS IN INFLAMMATORY DISEASE.

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Abstract

Asthma results from exposure to allergens and other airway irritants. While clinical treatment of the disease has recently expanded beyond simply β-agonists and inhaled steroids, recent strategies seeking to modulate the immune mechanisms of the disease have offered incomplete control of the disease. This study demonstrates a mechanistically novel component in the development of asthmatic inflammation mediated by sensory neurons within the lung parenchyma. Sensory neurons are rich in the transient receptor potential channel A1 (TRPA1), an ion channel activated by strong triggers of asthma, including cigarette smoke, chlorine, and hypochlorite. This project examines the role of TRPA1 in the development of allergic inflammation in an ovalbumin-based murine model of asthma. All quantifiable measures of asthmatic inflammation including airway hyperreactivity, mucus production, and cytokine production were significantly reduced by both genetic deletion and pharmacological blockade of TRPA1. This finding suggests not only that TRPA1 is a mechanistically important factor in the development of allergic inflammation, but it also offers a new potential pharmacological target in the treatment of asthma.

Acknowledgements

Initially, while impressed by the science, I decided to join the Jordt lab for a single reason: the people. The group I started with was phenomenal, and I learned very much from Christian von Hehn, Marian Brackmann, Jasmine Escalera, Bret Bessac and Aiwei Sui. Most of all, I appreciate Professor Jordt's scientific mentorship over the years of working together and his understanding during my shift of career paths. Equally, I appreciate Anabel Caceres' acceptance into her projects which has been a great learning opportunity and a lot of fun.

Numerous factors made my time devoted to this project possible. Primarily, the Yale System allowed me to explore my own academic interests without fear of compromising my medical school coursework. I sincerely hope that, despite current pressures, the school maintains its commitment to its students by allowing such freedom. Financially, I have been the recipient of generous financial awards from NIH training grants: 5T32GM07205 and 5T35HL07649. I am also grateful to the Taylor Opportunity Student Research Fellowship. These awards enabled me to dedicate more of my time to academic pursuits than would have otherwise been possible.

While working in the lab charged my mind scientifically, my time at Yale would not have been a happy period without my wife, Alex. She has brought joy to my life, and I will never forget the sacrifices that she continues to make to enable my career to progress. Thank you.

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Introduction

The rate of asthma in the United States and world-wide has increased significantly throughout the past decades [1, 2]. An estimated 9.2% of American children, and a global total of 300 million people, are affected by the condition [3], which is particularly concerning due to the potential for severe exacerbations that may be life-threatening.

The inflammatory response of asthma is characterized by a CD4 Th2 cell mediated infiltration of eosinophils, mast cell activation and, ultimately, tissue remodeling. Mucus hyperplasia and airway hyper reactivity are also characteristic [4]. Mast cells infiltrate the surrounding smooth muscle, [5] and the generalized inflammatory response eventually leads to airway remodeling, including smooth muscle hyperplasia and collagen deposition below the epithelial basement membrane [6].

Current treatment of asthma is largely focused on two classes of medications: bronchodilators and corticosteroids. These function to relax the smooth muscle of the airway as β_2-adrenergic agonists and through eliminating airway inflammation, respectively. Each of these medication classes carries with it associated side effects. β_2-adrenergic agonists produce dose-dependent tremor, tachycardia, and anxiety, and corticosteroids most commonly cause cough, vocal hoarseness, sore throat and predisposition to thrush. At high doses, corticosteroids can cause growth retardation [7], cataracts [8], elevated intraocular pressures, and bone loss [9]. Furthermore, corticosteroids merely suppress asthmatic inflammation. Discontinuation of steroids results in the return to baseline levels of asthmatic inflammation, eosinophilc infiltration, and bronchial hyperreactivity within 2 weeks [10]. While asthma related hospitalizations

and deaths in the United States have decreased over the past 30 years with the use of these and other medications [11], there remains room for improvement in current therapies. Novel approaches to the treatment of asthma focus on immune modulation, but these treatments are not considered first-line.

Novel drugs are needed to target and reduce inflammation. Sensory nerves within the lung parenchyma can mediate inflammation. Sensory neurons richly innervate the lung and are sensitive to noxious stimuli in the air [12], and recent evidence indicates that there is likely bidirectional feedback between immunogenic and neurogenic mechanisms of inflammation [13, 14]. Furthermore, neuronal activation induces many of the symptoms of asthma including inflammation, mucus hypersecretion, cough and bronchoconstriction [15, 16]. Nerve endings present in the nasal mucosa are the outermost detection point for noxious chemicals. Stimulation of these sensory neurons causes pain and sneezing [17]. These sensory nerves release neuropeptides including substance P and CGRP in response to noxious stimuli. These proinflammatory neuropeptides promote structural changes in the microvascular endothelium leading to edema formation and narrowing of the nasal airway [18, 19]. Stimulation of vagal sensory fibers in the glottis and larynx leads to coughing and the expulsion of noxious air. Irritants not expelled by the coughing reflex enter the lower airways and cause neurogenic inflammation, mucus secretion and airway hyperreactivity. In addition to these changes, inhaled irritants elicit other specific physiological responses. For example, rodents can decrease their respiratory rate by up to 50% in response to irritant exposure [20].

TRPA1 is a sensory ion channel expressed in approximately one-third of sensory neurons [21, 22]. While initial studies identified TRPA1 as the receptor for mustard oil (allyl isothiocyanate), further investigation identified additional agonists including cinnamic aldehyde, isovelleral, and diallyl sulfides [23-25]. Administration of any of these TRPA1 agonists to the skin causes pain, hyperalgesia and neurogenic inflammation [26, 27].

Early evidence suggests that TRPA1 is a possible airway irritant receptor. Prior to the identification of TRPA1, studies demonstrated that airway irritants led to calcium influx into sensory nerves [28]. Not only is TRPA1 a calcium-permeable channel, but pretreatment with ruthenium red, a non-specific Ca^{2+} channel blocker blocker, prevents bronchoconstriction in response to inhaled irritants [29-31]. Additionally, allyl isothiocyanate, a TRPA1 agonist, is a widely known upper airway irritant because it is the "active" ingredient of wasabi [32].

Following these preliminary suggestions of the role of TRPA1 in detection of airway irritants, Bautista et al. identified that acrolein, a highly irritating unsaturated aldehyde present in smoke, activated TRPA1[32]. Significantly, Trpa1-/- mice did not respond to this irritant by characteristically lowering their respiratory rates.

The role of TRPA1 in irritant detection expanded with the finding that it is responsible for detecting oxidants including ozone, hypochlorite, and chlorine gas [33]. This study found that neurons of the trigeminal and nodose ganglia, which innervate the lower airways, express TRPA1 and are responsible for irritant induced hyperreactivity. HC-030031, a TRPA1 selective antagonist, was subsequently found to eliminate neuropeptide-mediated bronchoconstriction in harvested guinea pig lungs in response to

exposure to unsaturated aldehydes [34]. HC-030031was shown in the same study to abolish neurogenic inflammation secondary to tobacco smoke. Taken together, these early studies have demonstrated that TRPA1 is responsible for detection of noxious stimuli including unsaturated aldehydes, hypochlorite, isocyanates and tobacco smoke. In response to these stimuli, TRPA1 activation leads to release of neuropeptides responsible for neurogenic inflammation and airway hyperreactivity.

Activation of TRPA1 is believed to occur via covalent modification of a cytosolic region of the channel [35, 36]. Known TRPA1 agonists, including unsaturated aldehydes, isothiocyanates, and oxidants are inherently reactive. Isothiocyanates likely form thiourea adducts with cysteine residues of the channel [35]. Unsaturated aldehydes react with cysteine residues through a Michael Addition and react with lysine and histidine residues to form Schiff bases. Oxidant agonists oxidize the thiol moiety of cysteines [37]. The finding that irreversible covalent modification of the channel can lock TRPA1 in an activated position allowed for the localization of the region of the channel sensitive to modification by an agonist. Using systematic mutagenesis replacing potential sites of covalent modification by allyl isothiocyanate with unreactive amino acids, a group of cysteine and lysine residues were identified at the cytosolic N-terminus that are responsible for channel activation [35]. Even though nearly every cytosolic cysteine residue was covalently modified during isothiocyanate treatment, blocking covalent modification at this specific region prevented activation [36]. Interestingly, the same residues are responsible for activation of TRPA1 by unsaturated aldehydes and oxidants [38-39]. Irreversible covalent modification and subsequent constant activation

of TRPA1 offers an explanation of the continued post-exposure irritation reported by individuals exposed to known TRPA1 agonists including tear gas and hypochlorite [39].

TRPA1 activity is not limited to exogenous activators. Oxidative stress produces numerous TRPA1 activators including reactive oxygen species (ROS), hypochlorite, and lipid peroxidation products. Oxidative stress, which produces these compounds, is present in airway inflammation caused by asthma, COPD, rhinitis, and viral infection [40-42]. Macrophages and neutrophils produce ROS during inflammation [43, 44] many of which activate TRPA1 [45]. For example, hypochlorite concentrations in the interstitial spaces during airway inflammation far the EC_{50} of TRPA1[39, 46]. In addition to the effects of ROS alone, these highly reactive compounds damage cell membranes creating lipid peroxidation products [47]. Lipid peroxidatioon products themselves are known agonists of TRPA1 in both heterologous expression systems and in vivo [45, 48-51]. One endogenous TRPA1 agonist with greatest in vivo effect has not been identified, and it is possible that a complex mixture of reactive species all activate TRPA1.

Taken together, this evidence suggests that the transient receptor potential (TRP) ion channel superfamily is involved in the response to inflammatory mediators [51-53]. Through TRP channels, sensory neurons are activated by ozone, chlorine, capsaicin, and acrolein. Each of these compounds has been linked to the development of reactive airway dysfunction syndrome [53-57]. Mustard oil, cinnamon oil, and wintergreen oil selectively activate TRPA1. Additionally, TRPA1 is activated by bradykinin through the action of a G-protein coupled receptor [58-60]. Moreover, reactive oxygen species and lipid peroxidation products are potentially significant endogenous activators of TRPA1

[61-63] making TRPA1 a likely player in the development of inflammation. In fact, blockade of TRPA1 reduces inflammatory hyperalgesia and decrease airway hyperreactivity in mice with chemically induced inflammation [55, 64].

Our work identifies TRPA1 as an important part of the development of experimentally-induced acute asthma. TRPA1 is important in inducing the release of neuropeptides, which leads to increased leukocyte infiltration, mucus hyper-production, and airway hyperreactivity. Animals with either genetically deleted or pharmacologically blocked TRPA1 do not generate the same response. Furthermore, while another member of the TRP superfamily, TRPV1, is a well-known proinflammatory channel expressed in sensory neurons, the development of experimentally induced asthma is not dependent upon this channel.

Specific Aims

1. Demonstrate the role of TRPA1 in the development of acute asthma in an OVA-based murine model

2. Demonstrate the ability of pharmacologic blockage or genetic deletion to reduce quantifiable measures of allergic inflammation including: airway hyperreactivity, mucus production, cell extravasation, and neuropeptide release.

Methods

Animals. Experimental procedures were approved by the Institutional Animal Care and Use Committees of Yale University, the University of California, San Francisco, and Hydra Biosciences. Mice were housed at facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care in standard environmental conditions (12-h light-dark cycle and 23 °C). Food and water were provided ad libitum. Trpa1-/- mice were a gift from David Julius (University of California, San Francisco). The Trpa1 knockout allele was backcrossed into the C57BL/6 background (>99.5%) by marker assisted accelerated backcrossing (Charles River Laboratories). Trpv1-/- mice were purchased from Jackson Laboratories and C57/Bl6 and BALB/c mice from Charles River Laboratories. For experiments on C57BL/6, Trpv1-/-, and Trpa1-/- mice, animals were matched for age (12–22 weeks) and gender. Six- to 8-week-old BALB/c mice were used for OVA sensitization and antagonist studies.

Measurement of Airway Reactivity. Twenty-four hours following the last OVA challenge, mice were anesthetized with pentobarbital (60 mg/kg of body weight) and urethane (1 g/kg). A tracheostomy was performed, and the trachea was cannulated by Dr. Caceres. Mice were attached to a Flexivent pulmonary mechanics analyzer (SCIREQ) and ventilated at a tidal volume of 9 mL/kg, at a frequency of 150 bpm. Positive end-expiratory pressure was set at 2 cm H_2O. Mice were paralyzed with pancuronium (0.1 mg/kg i.p.). A 27-gauge needle was used to administer acetylcholine (0.03, 0.1, 0.3, 1.0, and 3.0 mg/mL) through the subclavian vein to generate a concentration-response curve.

Measurements of airway mechanics were made continuously applying the single-compartment model.

Quantitative Real-time PCR. Lungs and DRGs were surgical removed and white cells were collected from BAL by centrifugation. CD4 Th2 cells were purified as previously described [65]. Tissue and cells were frozen in liquid nitrogen. Total RNA was isolated from tissue homogenates using RNeasy Mini Kit 50 (Qiagen). cDNA synthesis was performed with the High Capacity RNA-to-cDNA Kit (Applied Biosystems). Quantitative PCR was performed using TaqMan Gene Expression Assays (Applied Biosystems). Typically, 20-μL reactions contained 10 μL TaqMan Fast Universal PCR Master Mix (2X), 1 μL of the specific TaqMan assay, 1 μL cDNA, and water to 20 μL. Cycling parameters were 52 °C for 2 min for activation and 10 min initial setup at 95 °C, followed by 40 cycles of 95 °C for 15 s and 1 min at 60 °C (ABI 7500 Fast, Applied Biosystems). Each sample was run in triplicate and normalized to GADPH gene expression. C_T values were determined using ABI PRISM software and averaged. Relative quantification was determined by the $\Delta\Delta C_T$ method [66]. The TaqMan Gene Expression Assays included specific primers and FAM/MGB probes for mGAPDH [endogenous Control (4352932E)], mTRPA1 (Mm00625268_m1), mMucin 5 subtypes A and C (Mm01276735_m1), and mIL-5 (Mm99999063_m1).

Data Analysis and Statistics. Data were analyzed using Origin 8 (OriginLab Corp.) and SPSS (SPSS Inc.) software. Cell differentials and qPCR data were analyzed by Student's

t-test for independent samples. Airway forced oscillation data were analyzed by repeated-measures ANOVA, followed by Bonferroni post-hoc analysis.

Note: All animal handling, sensitization with OVA, and cytokine analysis was done by Dr. Caceres. Airway reactivity studies were a cooperative effort between Ana Caceres, Maxwell Elia, and Bret Bessac. Maxwell Elia performed all qPCR experiments.

Results

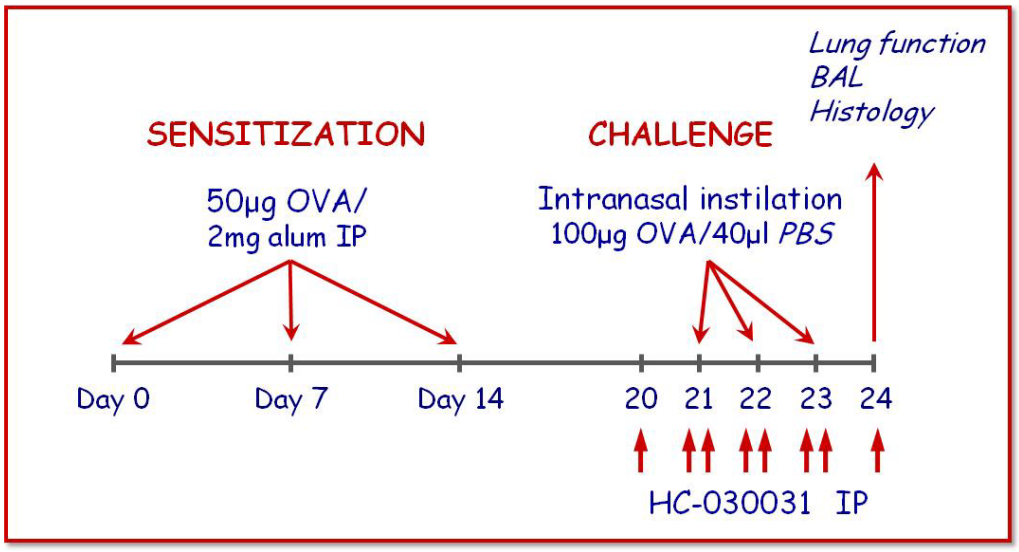


Figure 1: OVA Model of Allergic Asthma

Mice were sensitized with 50µg ovalbumin/2mg alum IP once per week for 3 doses. The mice were then given one week of rest after which they were given 3 intranasal administrations of 100µg ovalbumin/40µl PBS on consecutive days. Animals treated with HC-030031 were given 200mg/kg on day 20, and subsequently given 100mg/kg twice daily until sacrifice.

Diminished Leukocyte Infiltration in OVA-Challenged Trpa1-/- Mice.

Ovalbumin (OVA) was used to generate a Th2-directed allergic response. The OVA model was optimized by monitoring the leukocyte numbers in the bronchoalveolar lavage fluid (BALF) of wild-type C57BL/6 mice. Marked increases in eosinophils in BALF of wild-type mice were noted in response to OVA. This increase in response to OVA

challenge was blunted in Trpa1-/- mice. Trpa1-/- mice showed an 80% reduction in eosinophils in BALF. Interestingly, the cell counts of OVA challenged Trpv1-/- mice were indistinguishable from those of wild-type mice.

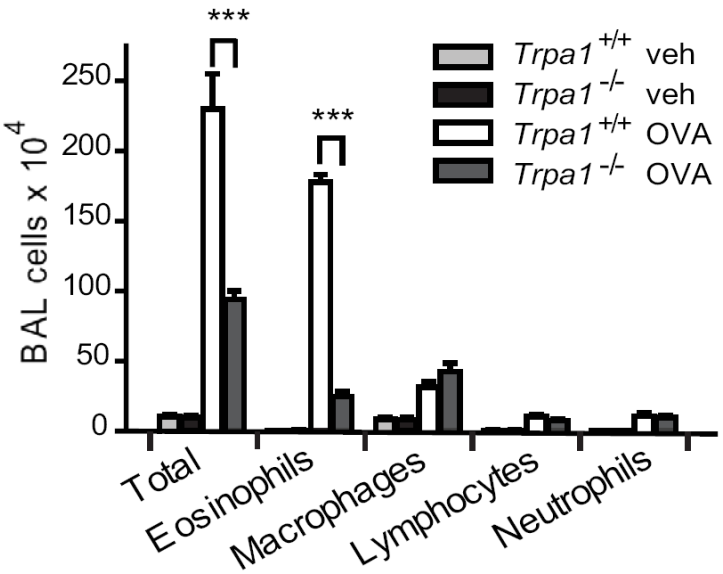


Figure 2: Reduced Cell infiltration in Trpa1-/- mice. Cell differentials from BALF from mice with and without OVA challenge. Animal groups: Trpa1+/+ OVA: n = 8, Trpa1-/- OVA: n = 8, Trpa1 +/+ veh: n = 7, Trpa1-/- veh: n = 6. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Diminished Airway Hyperreactivity in OVA-Challenged Trpa1-/- Mice.

Bronchoconstriction is a significant symptom of asthma. Forced oscillation plethysmography in response to i.v. administration of increasing concentrations of acetylcholine was used to assess airway hyperreactivity.

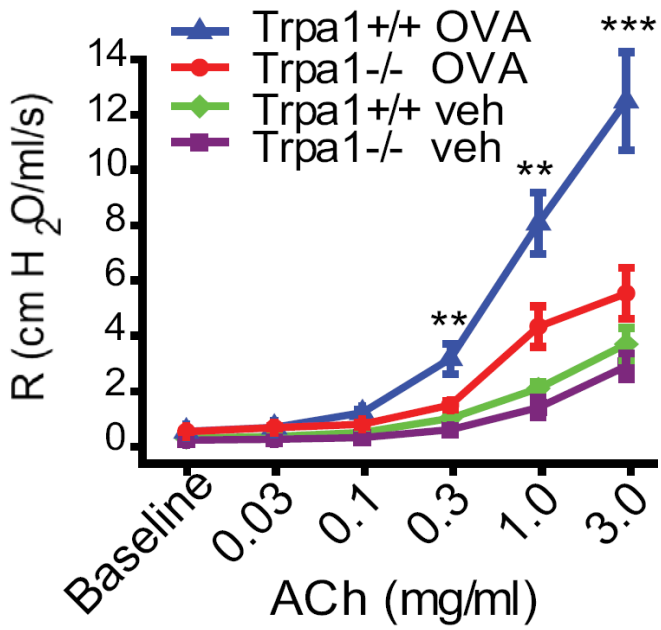


Figure 3: Reduced Airway Hyperreactivty in Trpa1-/- mice. Airway resistance as measured by Flexivent pulmonary mechanics analyzer. Animal groups: Trpa1+/+ OVA: n = 4, Trpa1-/- OVA: n = 4, Trpa1 +/+ veh: n = 7, Trpa1-/- veh: n = 6. (*, $\alpha = 0.05$; **, $\alpha = 0.01$; ***, $\alpha = 0.001$).

The difference in airway resistance between OVA-challenged Trpa1-/- was minimal compared to unchallenged mice. This confirms the necessary role of TRPA1 function in the asthmatic hyperreactivity.

Reduced Mucus Production in OVA-Challenged Trpa1-/- Mice.

Mucin is a widely expressed component of mucus, which is overproduced in asthmatics. Mucin transcription is a quantifiable measure of mucus hypersecretion, so mucin transcription was measured by qPCR to compare mice with and without OVA-challenge.

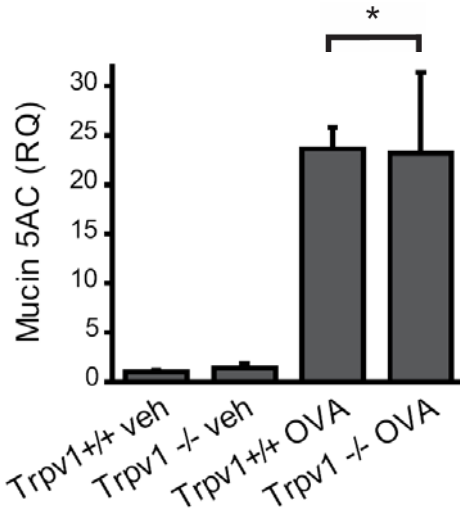


Figure 4: Impaired induction of Mucin Transcription in Trpa1-/-, but not Trpv1-/- mice in response to OVA-challenge. Relative quantities (RQ) of mucin5ac gene transcript, determined by qPCR of whole mouse lung cDNA. Mucin5ac induction is diminished in Trpa1-/- OVA mice. GAPDH transcript levels were used for normalization as endogenous control. Animal groups: Trpa1+/+ OVA: n = 4, Trpa1-/- OVA: n = 4, Trpa1 +/+ veh: n = 6, Trpa1-/- veh: n = 7. Trpv1 studies: n = 4 mice per group.
*, $P < 0.05$

Induction of mucus production as a result of OVA-challenge is blunted in the Trpa1-/- mice. In contrast, the expression mucin among Trpv1 -/- mice is the same as among wild-type mice further supporting the sole role of TRPA1 in the development of asthmatic inflammation.

Reduced Th2-Cytokine Transcription in Airways of OVA-Challenged Trpa1-/- Mice.

Interleukin 5 (IL-5) is a marker for Th2 mediated inflammation. Comparison of transcription of this cytokine allows for a meaningful, quantitative comparison of inflammation between the groups of mice. No transcriptional difference of IL-5 was noted between Trpa1-/- OVA-challenged mice and control animals whereas Trpv1-/- OVA-challenged mice had no such evidence of a blunted Th2 immune response.

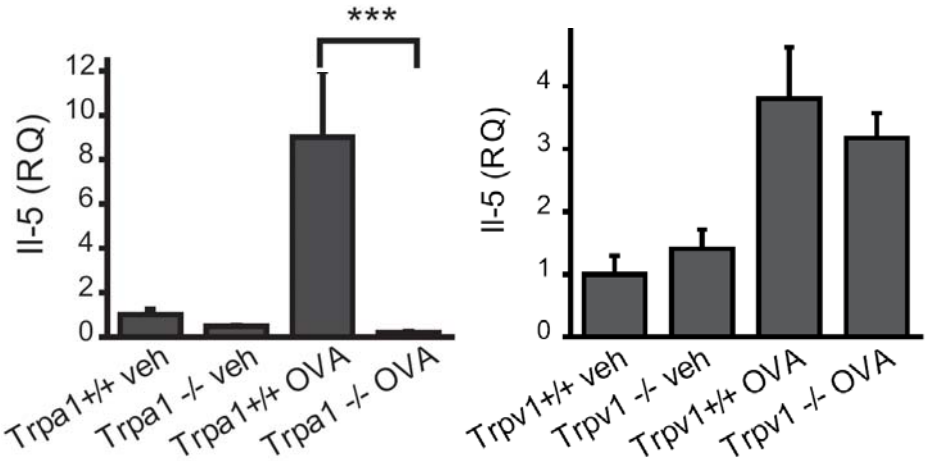


Figure 5: Decreased IL-5 transcription in Trpa1-/-, but not Trpv1-/-, mice in response to OVA-challenge. Relative quantities (RQ) of Interleukin 5 (IL-5) gene transcript, determined by qPCR of whole mouse lung cDNA. IL-5 is massively reduced in Trpa1-/- OVA-challenged mice. GAPDH transcript levels were used for normalization as endogenous control. Animal groups: Trpa1+/+ OVA: n = 4, Trpa1-/- OVA: n = 4, Trpa1 +/+ veh: n = 6, Trpa1-/- veh: n = 6. ***, $P < 0.001$. Trpv1 studies: n = 4 mice per group.

Previous ELISAs performed by Dr. Caceres found undetectable levels of INF-γ in all animal groups eliminating the possibility that the Trpa1-/- mice had a Th1 immune response as a result of the OVA-challenge.

TRPA1 Antagonist (HC-030031) Reduces Airway Hyperreactivity when Administered During OVA Airway Challenge.

HC-030031 has been shown to pharmacologically block TRPA1 and prevent inflammatory pain in animals treated with TRPA1 agonists [56, 64, 67]. HC-030031 was administered via intraperitoneal injection to Balb/C mice during the airway challenge phase of the OVA-model. The administration schedule of the drug was: the day before (200 mg/kg) and twice daily (100 mg/kg) during the 4 days of OVA airway challenge. Control mice were administered methyl cellulose (MC) on the same schedule. Similar to mice with genetic deletion of TRPA1, OVA-challenged Balb/C mice treated with HC-030031 show near elimination of lung hyperreactivity.

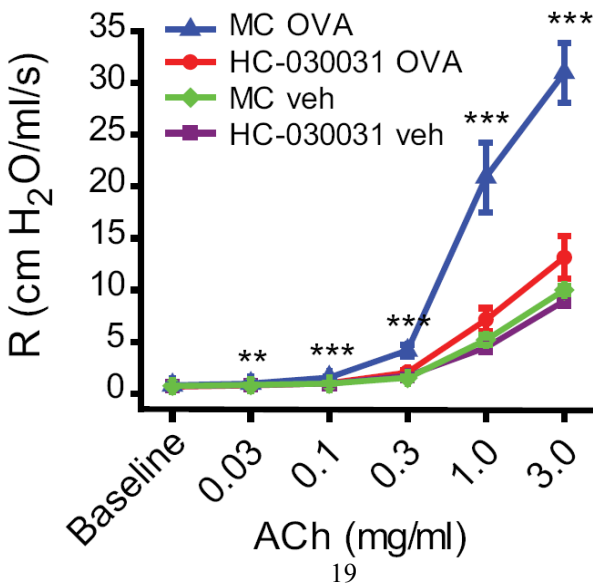


Figure 6: Reduced Airway Hyperreactivty in OVA-challenged mice treated with i.p. HC-030031.

Airway resistance as measured by Flexivent pulmonary mechanics analyzer for mice treated with either HC-030031 or with just methyl cellulose (MC). MC veh: n = 7, HC-030031 veh: n = 7, MC OVA, n = 7, HC-030031 OVA: n = 6

(**, $\alpha = 0.01$; ***, $\alpha = 0.001$)

Reduced Mucus Production in OVA-Challenged Balb/C mice treated with HC-030031.

Effects of HC-030031 were not limited to airway resistance. Similar to Trpa1-/- mice, animals treated with the TRPA1 antagonist showed a significant decrease in transcription of mucin by qPCR.

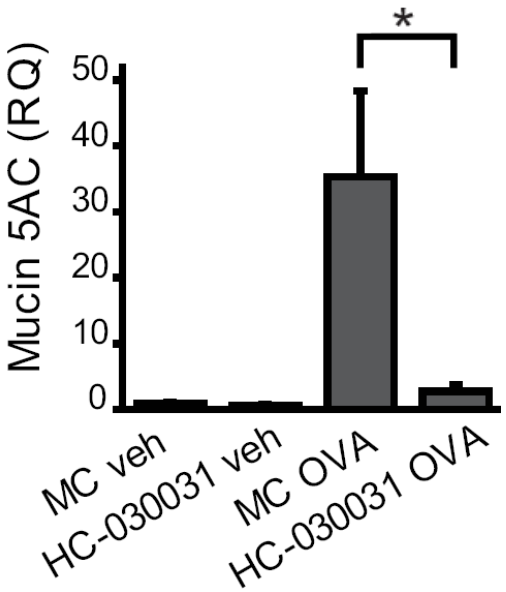


Figure 7: Impaired induction of Mucin Transcription Balb/C mice treated with HC-030031 during OVA-challenge. Relative quantities (RQ) of mucin5ac gene transcript, determined by qPCR of whole mouse lung cDNA. GAPDH transcript levels were used for normalization as an endogenous control. Animal groups: Trpa1+/+ OVA: n = 4, Trpa1-/- OVA: n = 4, Trpa1 +/+ veh: n = 8, Trpa1-/- veh: n = 8. *, $P < 0.05$.

Trpa1 proinflammatory action is neurogenic

To investigate the possibility that TRPA1 acts within immune cells, gene expression was evaluated in cDNA isolated from spleen, which includes a variety of immature immune cells, Th2 cells, whole mouse lung and in leukocytes isolated from BALF. Minimal transcription was noted except within DRG neurons further supporting the role of TRPA1 in neurogenic inflammation.

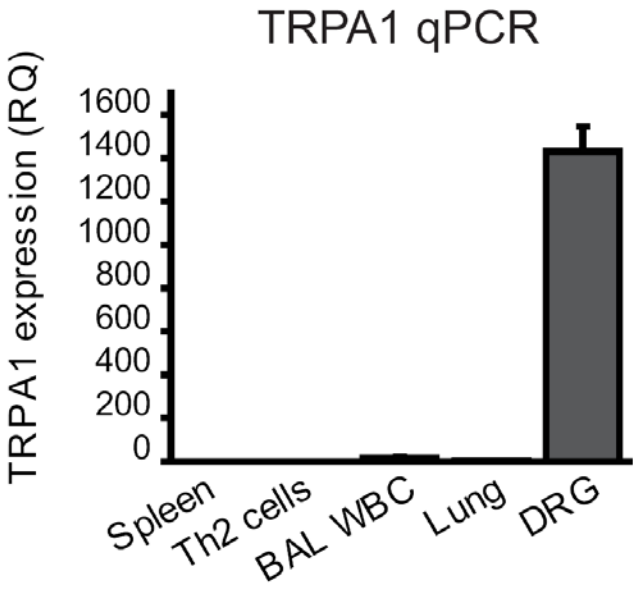


Figure 8: Absence of Tpra1 expression outside of sensory neurons

Relative quantities (RQ) of Trpa1 gene transcript, determined by qPCR of cDNA isolated from spleen , Th2 cells, leukocytes from BALF, whole lung, and DRG. GAPDH transcript levels were used for normalization as an endogenous control. Animal groups: n = 3-6 for all groups.

Additional studies were done with an Aspergillus model of asthma. This model, consisting of a total of 6 intranasal administrations of Aspergillus protein extract. The schedule of the model is summarized in the following figure.

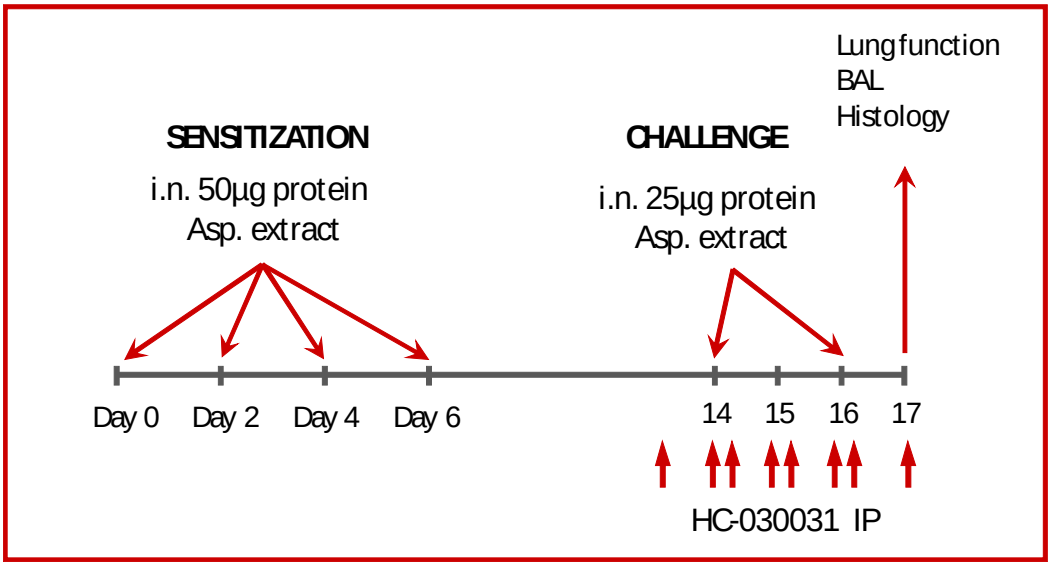


Figure 9: Summary of Aspergillus Model

Mice were given 4 intranasal administrations of 50µg protein/40µl PBS every other day. The mice were then given one week of rest after which they were given 2 intranasal administrations of 25µg protein/40µl PBS on alternate days. HC-030031 was administered starting with 160mg/kg on day 13 followed by 80mg/kg twice daily until sacrifice.

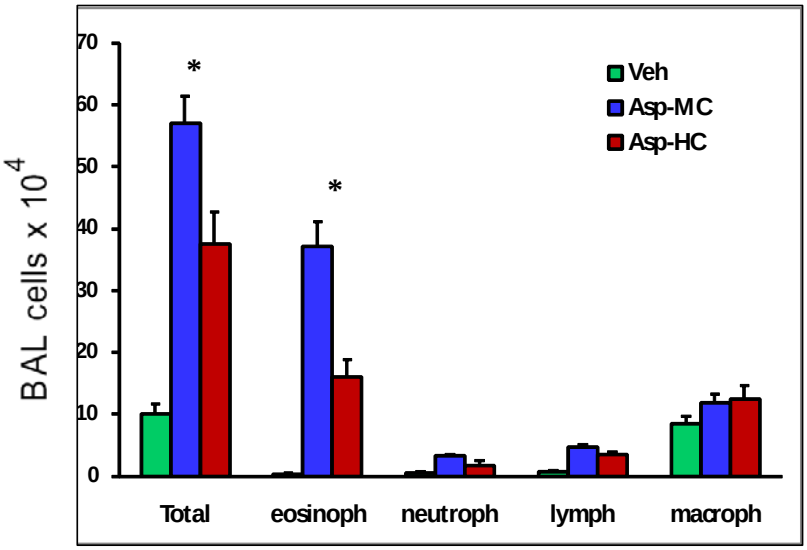


Figure 10: Diminished Leukocyte Infiltration in Aspergillus-Challenged Mice Treated with HC-030031. Cell differentials from BALF from mice with and without HC-030031 treatment demonstrating decreased eosinophilia. Animal groups: Veh: n = 6, Asp-MC: n = 4, Asp-HC: n = 4. *, $P < 0.05$.

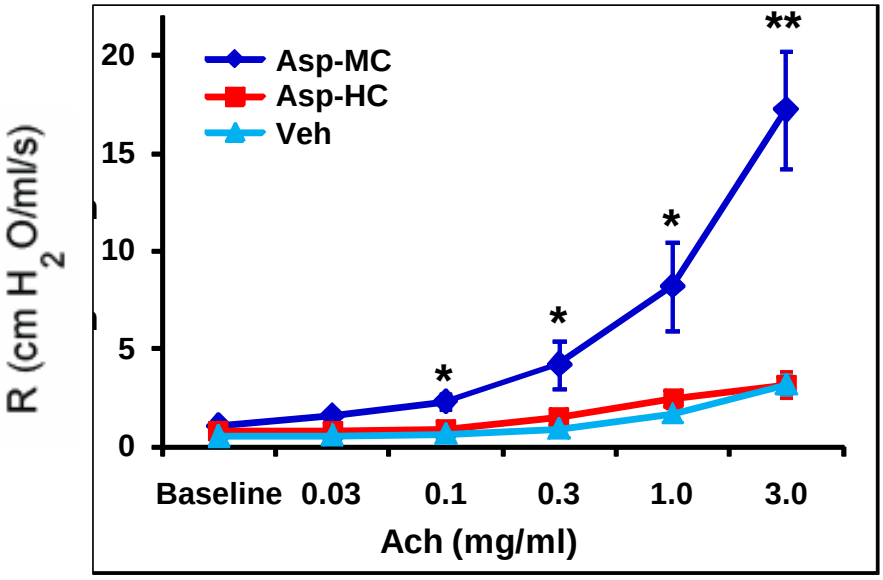


Figure 11: Treatment with HC-030031 reduced Aspergillus induced airway hyperreactivity. Airway resistance as measured by Flexivent pulmonary mechanics analyzer. Animal groups: Veh: n = 6, Asp-MC: n = 4, Asp-HC: n = 4. *p <0.05; ** p <0.01

Notably, treatment with HC-030031 reduced airway resistance to the level of mice which had never been exposed to Aspergillus.

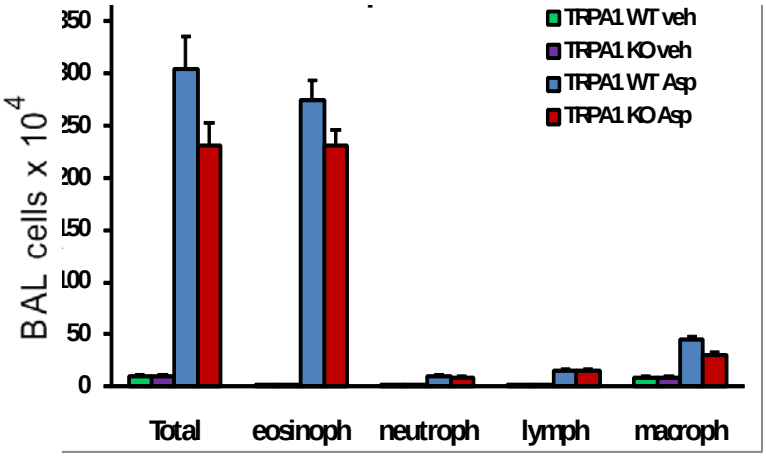


Figure 11: No significant difference in mice Diminished Leukocyte Infiltration in Aspergillus-Challenged Mice Treated with HC-030031. Cell differentials from BALF from mice with and without HC-030031 treatment demonstrating decreased eosinophilia. Animal groups: Veh: n = 6, Asp-MC: n = 4, Asp-HC: n = 4. *, $P < 0.05$.

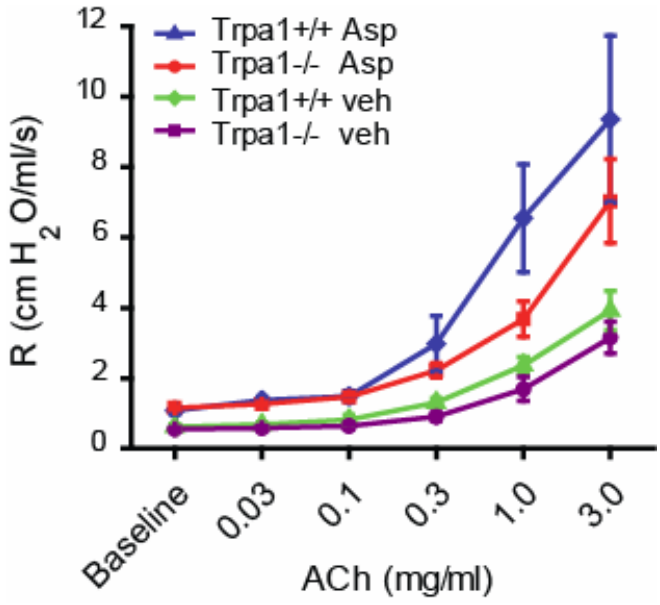


Figure 12: Reduced Airway Hyperreactivty in Trpa1-/- mice. Airway resistance as measured by Flexivent pulmonary mechanics analyzer. Trpa1+/+ Asp: n = 4, Trpa1-/- Asp: n = 4, Trpa1 +/+ veh: n = 4, Trpa1-/- veh: n = 4.

While there is no statistically significant difference between the groups, there is a strong trend showing reduced airway resistance among Trpa1 -/- mice.

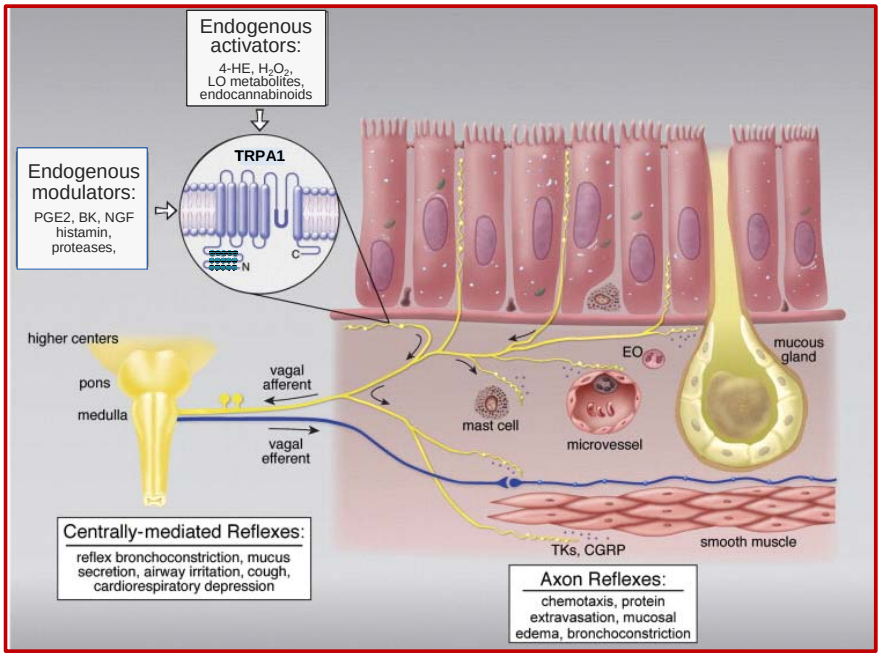
Discussion

These findings illustrate the role of TRPA1 in the development of asthmatic inflammation in an OVA albumin model of asthma. Preliminary findings suggest similar responses in an Aspergillus based model of asthma. Trpa1-/- mice showed diminished airway resistance, Th2 cytokines, and mucin synthesis compared to wild-type mice in an OVA model. In addition to Trpa1-/- mice, pharmacological blockade also reduces airway hyperreactivity, Th2 cytokines and mucin. Both small-molecule pharmacological blockade of TRPA1 and genetic ablation of the channel both produce reduced asthma susceptibility. This confirms that the anti-asthmatic response is due to inactivity of the channel itself, not developmental defects associated with genetic deletion of Trpa1.

TRPA1 is a sensor of both exogenous and endogenous pro-inflammatory molecules that are known to induce asthma. Exogenous activators include chlorine, unsaturated aldehydes smoke, tear gas agents, and industrial isocyanates [53-56, 67]. Endogenous activators, including reactive oxidative species and lipid mediators, are produced by infiltrating leukocytes and inflamed tissue and reach concentrations sufficiently high to activate TRPA1 [61, 62, 68].

The mechanism of the anti-inflammatory effect (Figure 8) of TPRA1 blockade is not certain, but is likely due to the reduction in the release of neuropeptides by sensory neurons. Activation of TPRA1 by endogenous activators causes the release of neuropeptides. These neuropeptides, including Neurokinin A, Substance P, and CGRP act to produce microvascular changes including endothelial cell contraction and upregulation of selectins and integrins. The expression of adhesion molecules, which are complimentary to those on circulating leukocytes, mediates migration of inflammatory

cells across the endothelium. Consequently, reduced activation of TRPA1 would diminish neuronal excitation and a decrease in Ca2+ influx into sensory neurons. Data from Dr. Marian Brackmann, not included here, found that levels of Neurokinin A, Substance P, and GCRP were all significantly decreased in OVA treated Trpa1-/- mice compared to OVA challenged wild type mice [69]. This finding is consistent with reduced sensory neural activation.



Modified from Lee & Gu; Curr Opin Pharmacol. 2009

Figure 13: Mechanism of Neurogenic Inflammation in Airways

Activation of TRPA1 by endogenous agonists leads to neuropeptide release which leads to subsequent microvascular leak, mucus hypersecretion and airway hyperreactivity.

Earlier work by Hegde and coworkers [70] highlights the significance of neuropeptides in the formation of lung inflammation. Substance P, through its action on the Neurokinin-1 receptor, acts to induce changes that allow for increased post capillary permeability, leukocyte infiltration and an increase in mucus secretions in the airways [71]. Their work identified that by pharmacologic blockade of the Neurokinin-1 receptor with SR140333 they could decrease measures of airway inflammation. They registered significant decreases in neutrophil extravasation as measured by MPO activity, cytokine release and the expression of cell adhesion molecules I-CAM1, V-CAM1, E-Selectin and P-Selectin in lung in a bacterial sepsis model.

Neuropeptide release is reduced in mice with either genetically or pharmacologically inactivated Trpa1, thereby reducing leukocyte infiltration and airway hyperreactivity. Importantly, this study assessed whether this result could have been due to the effects of TRPV1, another proinflammatory channel present in sensory neurons. Given that neither genetic inactivation nor pharmacologic blockade of TRPV1 reduces airway hyperreactivity, leukocyte infiltration, cytokine production or mucus production, it is clear that the development of asthmatic inflammation is solely due to endogenous activators of TRPA1 leading to an increase in the release of neuropeptides. While the development of asthmatic inflammation is not a TRPV1 dependent event, this channel may still be involved in the development of asthmatic symptoms secondary to airway inflammation.

Despite encouraging evidence that asthma related mortality is decreasing, asthma continues to pose a significant public health problem, and new methods of controlling the disease are needed. The finding that TRPA1 is essential for the development of

asthmatic inflammation in a murine model supports the idea that this channel represents a mechanistic bridge between chemical and immunological stimuli in the airways. This work also offers molecular insights into the pathway by which chemical exposures exacerbate asthma. The identification of the role of TRPA1 as an important mediator of asthmatic inflammation provides a novel pharmacologic target for the treatment of asthma. While current TRPA1 antagonists are likely poor drug candidates for the treatment of asthma because of their poor bioavailability, different formulations of this class of compound, or chemically modified derivatives, may provide needed relief to the asthmatics of the future.

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