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Allergic asthma: RIPK2 takes the lead

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Asthma affects more than 300 million people worldwide with estimations that about 7% Americans currently suffer from the disease.¹ The prevalence of asthma has increased exponentially over the past few decades highlighting the need for novel therapeutic interventions. Allergens including house dust mite (HDM) are the common cause of allergic asthma that affect 1–2% of the world's population.² The current treatment strategies for asthma include beta-agonists (bronchodilator) and corticosteroids (immune suppressant).³ Leukotriene inhibitors Montelukast and Theophylline are used for controlling chronic asthma, whereas biologics such as anti-IgE and anti-IL-5 are recommended for severe and specific cases of asthma where other strategies have failed.³ Other biologics such as anti-thymic stromal lymphopoietin, anti-IL-4/IL-13 are in clinical trials.³ Given the mere number of biologics that have been approved and are in clinical trials; the need for new treatment strategies are highly warranted.

Cytoplasmic sensors that include Nod-like receptors (NLR) are relatively new classes of pathogen-recognition receptors that guard our cellular sanctity by responding to both pathogen- and damage-associated molecular patterns.⁴ Several studies have shown an important role for these NLRs in regulating lung inflammation.⁵ However, the role of these cytoplasmic sensors in the development of allergic asthma remain poorly understood. In the current issue of *Journal of Leukocyte Biology*, Miller et al.⁶ demonstrate a central role for receptor-interacting protein kinase 2 (RIPK2) in promoting lung inflammation and allergic asthma in mice following sensitization with HDM (*Dermatophagoides farinae*), which is a common source of household allergen (Fig. 1).

Recent studies have reported conflicting results regarding the role of RIPK2 in allergic airway inflammation. siRNA-mediated deletion of lung RIPK2 in BALB/c mice during OVA-induced asthma provide significant protection when compared with wild-type (WT)

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DISCLOSURE

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BALB/c controls.⁷ In contrast, *Ripk2*^{-/-} mice on a C57BL/6 background show similar susceptibility to OVA-induced asthma when compared with C57BL/6 WT controls.⁸ While differences in genetic background of mice could account for the observed differences in the function of RIPK2 during OVA-induced asthma; Miller et al.⁶ used HDM-induced asthma as a mouse model system to resolve the role of RIPK2. To induce airway inflammation and asthma, mice are intratracheally sensitized with 60 μ g HDM on day 0 followed by daily HDM challenge (37.5 μ g intratracheally) from days 7 to 11. When analyzed on day 14, 3 days after the last HDM challenge, WT mice show significant airway pathology as evidenced by airway inflammation/cellular infiltration, bronchial lumen narrowing, and epithelial layer thickening in H&E sections; significant mucus production via PAS staining; and significant collagen deposition via Trichrome staining. Importantly, all HDM-induced airway pathologies are significantly attenuated in *Ripk2*^{-/-} mice. Overall, *Ripk2*^{-/-} mice show significant protection from HDM-induced airway inflammation when compared with C57BL/6 controls suggesting a central role for RIPK2 in driving inflammation and pathogenesis during asthma.⁶ Nucleotide oligomerization domain (NOD) protein 1 and 2 (NOD1 and NOD2) are the cytoplasmic sensors that recruit RIPK2 to initiate signaling and so far, receptors other than NOD1 and NOD2 have not been described for RIPK2.⁹ Studies comparing HDM-induced asthma in WT, *Ripk2*^{-/-}, *Nod1*^{-/-}, and *Nod2*^{-/-} mice will elucidate whether NOD1, NOD2, or both receptors are involved in the sensing of HDM to induce RIPK2-dependent allergic asthma. A positive observation will set up future experiments to further elucidate the potential HDM components that engage and activate the NOD receptor.

Even though RIPK2 was originally identified as a serine/threonine kinase, recent studies have established RIPK2 as a dual specificity kinase that can also phosphorylate tyrosine residues.¹⁰ Moreover, the kinase function of RIPK2 has not been well studied with most of the RIPK2 function in NOD1 and NOD2 signaling attributed to RIPK2 scaffold activity. Miller et al.⁶ demonstrate that HDM extract (250 PNU/ml of *D. farina*) stimulation of human airway epithelial cell lines Calu3 and A549 induced acute RIPK2 tyrosine phosphorylation in vitro, suggestive of RIPK2 activation. However, the requirement of RIPK2 phosphorylation for RIPK2 function and activity needs further investigation. Importantly, future studies will need to examine the contribution of RIPK2 kinase activity versus scaffold function in promoting HDM-induced airway inflammation. Studies examining HDM-induced airway inflammation in control, *Ripk2*^{-/-} and *Ripk2*^{KD/KD} (kinase dead) mice will reveal the potential involvement of RIPK2 kinase activity in promoting allergic asthma. If RIPK2 kinase activity is confirmed to be important, an FDA approved drug Ponatinib, which is shown to inhibit RIPK2 phosphorylation could provide an alternative to treat allergic asthma.¹¹

Airway epithelial cells are constantly exposed to allergens including HDM and as such play an important role in maintaining airway homeostasis. Miller et al.⁶ propose that RIPK2 functions in the airway epithelial cells to regulate early chemokine/cytokine production that in-turn promotes Th2 immune responses during HDM exposure. HDM extract stimulation of human airway epithelial Calu3 cells induce robust expression of *CCL2* mRNA, which is abolished in Calu3 cells with CRISPR/Cas9-mediated knockdown of RIPK2.⁶ Further corroborating these findings, HDM extract-induced *Ccl2* mRNA expression and cytokine

production from WT primary murine tracheal epithelial cells (MTECs) are abolished in *Ripk2*^{-/-} MTECs.⁶ Finally, to determine RIPK2's role in HDM-induced CCL2 production in vivo, mice were intratracheally challenged with 60 μ g HDM and lungs harvested 1 day later. HDM induced robust *Ccl2* mRNA and CCL2 protein expression in the lungs of WT mice but not *Ripk2*^{-/-} mice.⁶ Altogether, these data strongly suggest a role for RIPK2 in epithelial cells in promoting acute CCL2 production during HDM stimulation. In addition to CCL2, *Ripk2*^{-/-} mice also produce reduced levels of KC, TARC and MIP-1 β which could contribute to the overall inflammation and priming of Th2 responses.

Previous studies have shown that CCL2 promote myeloid cell infiltration and Th2 polarization.¹² Thus, it is possible that the CCL2 produced by HDM-stimulated epithelial cells is instrumental in shaping a Th2 biased milieu to promote airway inflammation and asthma. To address whether acute CCL2 is important in provoking HDM-induced airway inflammation, future studies should use CCL2 neutralizing antibodies during HDM sensitization, which should halt the progression of disease following HDM challenge. *Ccl2*-deficient mice are readily available and comparing the HDM-induced pathologies of *Ccl2*^{-/-} mice with *Ripk2*^{-/-} mice should provide definitive answers regarding the role of CCL2 in allergic asthma.

How does acute production of RIPK2-dependent CCL2 production by epithelial cells instruct a Th2-biased immune response to promote airway inflammation and asthma? What are the cell types that directly receive and integrate CCL2 signals? Experiments examining the expression of CCL2 receptor CCR2 in various immune cell types in the lungs and draining lymph nodes should provide critical insight on CCL2–CCR2 signaling axis. Miller et al.⁶ show that HDM challenge increases the total number of immune cells in the bronchoalveolar lavage (BAL) and the lung tissues of WT mice, which are significantly reduced in *Ripk2*^{-/-} mice. Further analysis of specific cellular populations by flow cytometry demonstrate that the total numbers of both eosinophils and neutrophils are significantly reduced in the BAL of *Ripk2*^{-/-} mice, whereas only eosinophils are reduced in the lung tissues.⁶ Namely, both lymphocytes and alveolar macrophage populations are similarly induced in WT and *Ripk2*^{-/-} mice.⁶ Despite similar recruitment to the BAL and lung tissues, *Ripk2*^{-/-} lymphocytes are functionally different from WT lymphocytes.⁶ In the spleen and draining lymph nodes of HDM-challenged mice, ELISPOT assay detect significantly reduced numbers of IL-4, IL-5, and IL-17 producing *Ripk2*^{-/-} T cells when compared with WT T cells.⁶ Flow cytometry analysis confirmed that CD4 T cells from the lungs of HDM-challenged *Ripk2*^{-/-} mice produce significantly lower amounts of IL-4, IL-5, and IL-17 cytokines when compared with WT controls.⁶ Finally, IL-4 and IL-5 cytokines detected in the lung homogenates from HDM-treated WT mice are significantly reduced in HDM-treated *Ripk2*^{-/-} mice.⁶ In addition to T cells cytokines, the production of IgG1 (Th2 associated antibody) by B cells is reduced in the serum of HDM-treated *Ripk2*^{-/-} than HDM-treated WT controls.⁶ Interestingly, IgE levels in the serum of HDM-treated mice are similar between WT and *Ripk2*^{-/-} mice. Alveolar macrophages and innate lymphoid cell (ILC2) cells are similarly induced in the lungs of HDM-treated WT and *Ripk2*^{-/-} mice as determined by flow cytometry, although functional studies will elucidate whether cytokine production by ILC2 are dependent on RIPK2. RIPK2 has been shown to play an important role in the dendritic cells to regulate adaptive immune responses¹³ and could potentially

have an important role in HDM-induced asthma as well, which will need further investigation.

Given the role of RIPK2 as a central adaptor that provokes HDM-induced allergic asthma, it will be important to identify the specific cell types within which RIPK2 is functional. Evidence provided by Miller et al.⁶ suggest that RIPK2 may be important in lung epithelial cells, but do not exclude the role of RIPK2 in other immune cells that promote allergic asthma including eosinophils, ILC2, macrophages, DC, T cells, and B cells. Bone marrow chimera studies of WT and *Ripk2*^{-/-} mice will determine the hematopoietic versus non-hematopoietic nature of RIPK2 in promoting allergic asthma. To identify specific cell types, it will be necessary to generate *Ripk2*^{fl/fl} mice, whereby RIPK2 can be conditionally deleted in specific cell types.

Overall, the key findings made by Miller et al.⁶ clearly demonstrate a central role for RIPK2 in establishing a Th2 immunity against HDM, that eventually promotes airway inflammation and asthma in mice. Mechanistically, HDM engages and activates RIPK2 through a yet unknown pathway (but likely involve NOD1 or NOD2) to induce early CCL2 production by lung airway epithelial cells to recruit and initiate Th2 immunity. Given the exciting observations and outstanding questions, the long-term goals of these projects should be to examine the basic fundamental questions that will not only help understand the etiology and biology of allergic asthma but also the biology of HDM. How does HDM hijack RIPK2 machinery to promote airway inflammation? Can we identify specific ligands or moieties from HDM that promote RIPK2 signaling? Is RIPK2-dependent Th2 immune responses observed in HDM-mouse model specific to a particular HDM in general? OR is this pathway applicable to a wide range of stimuli that are known to activate RIPK2 signaling? All these queries will undoubtedly open up new areas of research and identification of key molecular pathways both in the host and the allergens that may one day be targeted for treatment of asthma.

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Abbreviations:

BAL	bronchoalveolar lavage
HDM	house dust mite
ILC	innate lymphoid cell
MTEC	murine tracheal epithelial cells
NLR	nod-like receptors
NOD	nucleotide oligomerization domain
RIPK2	receptor interacting protein kinase 2

Th	T helper
WT	wild-type

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FIGURE 1. Cellular and molecular events during HDM-induced allergic asthma in mice. Mice are challenged and sensitized on several days with HDM intratracheally to induce airway inflammation that mimic allergic asthma. HDM are sensed by airway epithelial cells (hypothesized to be through either NOD1 or NOD2) whereby RIPK2 is tyrosine phosphorylated and activated, to promote CCL2 production. The early production of CCL2 is crucial for recruitment of inflammatory cells into the lung milieu. CCL2 through yet unknown mechanisms promote recruitment of both innate (eosinophils, neutrophils) and adaptive (T cells, B cells) immune cells into the lungs and lung draining lymph nodes. It is

not clear whether CCL2 instructs these immune cells to promote a Th2 responses, or whether RIPK2 has cell intrinsic function within these immune cells to drive Th2 responses. Regardless, following HDM sensitization and challenge, RIPK2-dependent Th2 responses dominate the lung which promotes allergic airway inflammation in mice.

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