

When It Comes to *Alternaria*, Instant CARMA's Gonna Get You

The airway epithelium comprises a continuous cell layer lining both large and small airways, serving as the first line of defense against inhaled pathogens and environmental factors such as allergens and air pollution particulates (1–3). Increasingly, these airway structural cells are being appreciated as active rather than passive players in the immune response to airway injury, particularly in the context of allergic asthma. Airway epithelial cells (AECs) actively participate in orchestrating both innate and adaptive immune responses through cellular signaling and interactions with inflammatory and dendritic cells (3). In response to damage from inhaled irritants, AECs release alarmins such as IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) to drive type 2 immune responses. These cytokines stimulate dendritic cell maturation and activate type 2 innate lymphoid cells and T-lymphocytes to release type 2 cytokines (IL-4, IL-13, and IL-5) upon allergen exposure (4, 5). Secretion of these airway epithelium-derived cytokines is significantly increased in allergic asthma and is inversely associated with FEV₁% predicted in patients with asthma (6).

In this issue of the *Journal*, Causton and colleagues (pp. 684–694) offer insight into a mechanism of innate immunity coordinated by AECs in response to an inhaled fungal allergen (7). Caspase recruitment domain-containing membrane-associated guanylate kinase protein 3 (CARMA3) is an intracellular scaffold protein that mediates G-protein-coupled receptor (GPCR)-induced signaling in AECs (8, 9). Previous studies have shown that CARMA3 is highly expressed in human and murine AECs, where it augments NF- κ B activity, and TSLP and CCL20 expression, in response to stimulation with the GPCR agonist lysophosphatidic acid (9). In addition, CARMA3 mediates proinflammatory cytokine release from normal human bronchial epithelial (NHBE) cells after stimulation with allergens such as *Alternaria alternata* and house dust mite, both of which activate the GPCR protease-activated receptor 2. Mice with targeted disruption of *Carma3* expression in AECs that were subjected to T-cell-dependent models of allergic asthma exhibited reduced TSLP, type 2 cytokine production, and airway eosinophilia, but no attenuation of airway hyperresponsiveness (10). Furthermore, these mice demonstrated impaired dendritic-cell maturation and T-cell proliferation. The current study by Causton and colleagues extends those studies by showing that airway epithelial expression of *Carma3* is essential for initiation of an innate, T-cell-independent immune response to *A. alternata* (7). They observed that pulmonary gene expression of epithelial cytokines, such as *il25* and *il33*, was reduced after a 4-day challenge with *A. alternata* in mice deficient for *Carma3* in AECs. They noted reduced alveolar levels of IL-33 protein in these mice, which in turn led to a reduction of type 2 innate lymphoid cell activation and consequent reduced allergic inflammation. The authors also found that knockdown of CARMA3 expression in NHBEs reduced the rapid (within 30 min) release of preformed IL-33 in response to *A. alternata*, suggesting a non-NF- κ B-mediated CARMA3 signal. *A. alternata* is known to stimulate calcium flux in the epithelium, and interestingly, this intracellular calcium flux was delayed and attenuated with CARMA3 knockdown expression in

NHBE cells. CARMA3 was also shown to colocalize with inositol 1,4,5-triphosphate receptors (ITPRs) in AECs, indicating that CARMA3 may mediate allergen-stimulated calcium mobilization in these cells, distinct from (and likely in parallel with) its activation of NF- κ B (7). Although other CARMA family proteins have been shown to activate divergent downstream signaling pathways, this is the first demonstration of CARMA3-dependent activation of a rapid calcium-mediated signal cascade, in addition to its activation of an NF- κ B-mediated inflammatory response (11).

Although the observation of attenuation of calcium flux by CARMA3 disruption and the association between CARMA3 and ITPRs is notable, the authors have not proven that this protein-protein interaction directly modulates calcium flux. Such proof would depend on identifying and modifying the sites of interaction between CARMA3 and any of the various ITPRs to assess the functional impact. Furthermore, the direct impact of calcium flux on triggering the secretion of cytokines remains undetermined, especially in light of the authors' finding that IL-33 secretion from CARMA3 knocked-down NHBE cells was not attenuated at later time points. Indeed, as an intracellular scaffolding protein, CARMA3 may be critical for mediating the formation of preformed pools of IL-33, distinct from its impact on gene expression or NF- κ B signaling. On the other hand, *A. alternata* exposure may activate epithelial signaling cascades that do not depend on CARMA3 and rapid calcium fluxes. In addition, NF- κ B activation itself is sensitive to calcium flux (12). Although the authors correctly assert that the rapid release of IL-33 in response to *A. alternata* likely reflects calcium flux and not NF- κ B signaling, it remains quite possible that NF- κ B signaling would still be impacted by the altered calcium flux (separate from the lack of CARMA3 signaling). Finally, the authors confirm an intriguing observation from their previous work: that airway hyperresponsiveness and type 2 inflammation can be dissociated through mutation of *Carma3*. Understanding the triggers for airway hyperresponsiveness under conditions of reduced CARMA3-dependent calcium flux and type 2 cytokine secretion may have broader implications for our understanding of nonallergic asthma.

In summary, the authors have demonstrated that CARMA3 is critical for innate responses to *A. alternata* and in adaptive models of allergic airways inflammation. In addition, they suggest a novel mechanism whereby CARMA3 regulates rapid calcium flux in response to *A. alternata*, possibly through interactions with ITPRs. These data further strengthen the importance of the airway epithelium as a critical player in directing and defining the allergic inflammatory response, and also reveal a novel therapeutic target with importance both in the early stages of allergic asthma (when innate responses predominate) and in the later, adaptive stages of allergic airway inflammation. ■

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