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Competing interests

The authors declare no competing interests.



ASTHMA & ALLERGY

A soluble allergen sensor sounds the alarm

The identification of the acute phase protein serum amyloid A as a soluble allergen sensor sheds new light on the mechanisms involved in the induction of type II airway inflammation.

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The airway epithelium forms a tight barrier to the outside world and plays a central role in the pathogenesis of allergic asthma and rhinitis¹. A growing body of evidence indicates that many allergens can directly engage epithelial pattern recognition receptors (PRRs). These PRRs should be able to discriminate between harmful and innocuous substances but, in susceptible individuals, sensing of inhaled allergens by epithelial cells is thought to be dysregulated. As a result, PRR engagement by allergens evokes the epithelial secretion of chemokines, innate cytokines and alarmins, culminating in a robust type II adaptive immune response. However, the molecular mechanisms by which allergens stimulate PRRs and thereby induce the recruitment and activation of critical players in type II inflammation remain largely undefined. In this issue of *Nature Immunology*, Smole and colleagues² demonstrate that the acute phase protein serum amyloid A (SAA) acts as a soluble innate environmental sensor that promotes pulmonary type II inflammation. The authors discovered that the interaction between SAA and allergens of the fatty acid binding protein (FABP) family, which includes the house dust mite (HDM) allergen Der p 13, drives the active release of interleukin (IL)-33 by epithelial cells. Their findings reveal a surprising and previously unrecognized role for SAA, identifying a new mechanism of allergen sensing that provides insight into how the expression of IL-33, a crucial innate cytokine in allergic inflammation, is induced.

Allergen sensitization is a critical step in the development of allergic asthma. Allergens are derived from complex living organisms and comprise a diverse group of proteins with unique molecular structures

and biological functions. It is therefore not surprising that many different mechanisms exist for the recognition of these allergens. Remarkably, all of these distinct pathways converge to induce eosinophilic airway inflammation. Allergens derived from the HDM species *Dermatophagoides pteronyssinus* are a major cause of asthma worldwide. HDM fecal particles contain a composite mixture of more than twenty mite-derived immunostimulatory protein groups, as well as endotoxin and proteases³. HDM allergens are strong activators of airway epithelial cells and innate immune cells because they contain specific lipid and carbohydrate ligands that can be recognized by a variety of PRRs. HDM-derived serine proteases, which interact with protease-activated receptor PAR-2, and the cysteine protease Der p 1 induce epithelial cytokine production and facilitate transepithelial delivery of allergens by disrupting tight junctions (Fig. 1). Another major HDM allergen, Der p 2, promotes airway inflammation via Toll-like receptor (TLR) 4, resulting in epithelial production of IL-1 α , which then triggers granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-33 release in an autocrine fashion⁴ (Fig. 1). Together, these proinflammatory signals trigger the activation of group 2 innate lymphoid cells (ILC2s) and dendritic cells (DCs), driving a T helper 2 (T_H2)-mediated response. Airway epithelial cells do not only express membrane-bound or cytoplasmic PRRs but are also a major source of soluble PRRs, such as SAA. Interestingly, SAA is known to bind to the bacterial outer membrane protein A (OmpA), which shares structural homology with the FABP Der p 13, a minor HDM allergen. This, together with the published finding that SAA is significantly increased in the blood and

sputum of patients with asthma, prompted Smole and colleagues to explore the role of SAA in allergic airway disease.

SAA, originally identified as a protein deposited in amyloid A-type amyloidosis in the early 1970s, is a major acute-phase protein that is present in the circulation bound to high-density lipoprotein. Its production in the liver is upregulated in response to trauma and systemic infection, resulting in an up to 1,000-fold increase in its serum concentration. SAA serves as an innate immune opsonin for Gram-negative bacteria and blocks virus entry into cells. Locally produced lipid-free SAA has been implicated in the recruitment of immune cells and in epithelial repair and has cytokine-inducing properties⁵.

Using mouse models, the authors provide convincing evidence that the interaction of SAA with allergens of the FABP family is required for the induction of HDM-mediated allergic airway inflammation. They show that targeted deletion of the *Saa* gene, which results in the loss of expression of SAA1 and its less abundant isoform SAA2, or SAA neutralization with specific antibodies diminishes the hallmarks of the HDM-driven allergic inflammatory phenotype. Compared with HDM-exposed wild-type mice, *Saa*^{-/-} mice displayed significantly reduced numbers of DCs, IL-13⁺ T cells and IL-13⁺ ILC2s in the lung. Bronchoalveolar lavage eosinophilia, goblet cell hyperplasia, total serum immunoglobulin E (IgE) and bronchial hyperresponsiveness were also diminished. Furthermore, Smole and colleagues established that SAA can interact with FABPs of arthropod group 13 allergens and that depletion of Der p 13 impaired the capacity of HDM extract to induce IL-33 release in bronchoalveolar lavage fluid

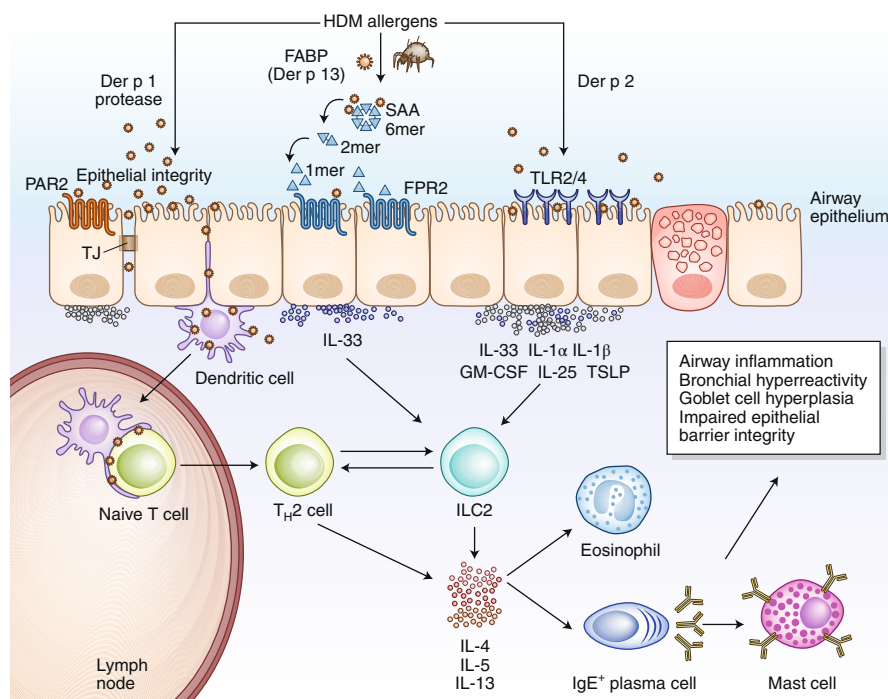


Fig. 1 | Induction of type II inflammation by various HDM-derived allergens. Allergen triggering of pattern recognition receptors (PRRs) induces activation of epithelial cells. Der p 1 is a cysteine protease that, together with various serine proteases (Der p 3, Der p 6 and Der p 9) that activate the protease-activated receptor 2 (PAR2), has the capacity to break down epithelial tight junctions (TJ). Der p 2 activates Toll-like receptor (TLR)2/4, leading to production of the indicated innate cytokines by epithelial cells. Smole and colleagues have identified a new pathway, dependent on the capacity of the fatty acid binding protein (FABP) allergen Der p 13 to induce dissociation of hexamers of the soluble PRR serum amyloid A (SAA), which results in epithelial IL-33 production via formyl peptide receptor-2 (FPR2) signaling. These epithelial proinflammatory signals induce the activation of group 2 innate lymphoid cells (ILC2s) and dendritic cells, which migrate to draining lymph nodes to present allergens to T cells, resulting in the differentiation of T helper 2 (T_H2) cells that migrate to the lung. In the ensuing allergic airway inflammation, cardinal features of allergic asthma are attributed to cytokines that are produced by ILC2s and T_H2 cells: IL-4 promotes class switching of allergen-specific B cells to IgE, IL-5 recruits eosinophils and IL-13 provokes goblet cell hyperplasia, mucus hyperproduction and smooth muscle hyperreactivity and impairs epithelial integrity. GM-CSF, granulocyte-macrophage colony-stimulating factor; TSLP, thymic stromal lymphopoietin.

in vivo³. These observations imply that strong HDM-derived allergens — including Der p 1 and Der p 2 — fail to induce innate immune activation in the absence of an interaction between FABP and SAA. Interestingly, only FABPs and none of the other mite allergens tested induced IL-33 release in the epithelial cell line BEAS-2B. Because IL-33 is known to increase the expression of components of the TLR4–MD-2–CD14 complex and the Myd88 adaptor, which signals downstream of many TLRs⁶, it is attractive to speculate that FABP–SAA interactions may be an early critical step that enhances innate sensing of HDM allergens via IL-33 release.

Smole and colleagues next employed the BEAS-2B cell line to explore how the structure of SAA relates to its ability to

interact with FABPs². HDM exposure did not affect the expression or release of SAA but led to the rapid dissociation of hexameric SAA into dimers and monomers (Fig. 1). Given that SAA is a retinol-binding protein that transports retinol during bacterial infection, the authors speculate that FABPs have the ability to displace small lipid molecules that stabilize SAA hexamers, resulting in SAA dissociation and the exposure of IL-33-triggering epitopes. This notion is supported by the finding that BEAS-2B cells increased the release of IL-33 (i) in the presence of antibodies that interfere with SAA hexamerization, (ii) when a point mutation in the hydrophobic core of SAA was introduced or (iii) when lipophilic molecules were depleted from the culture medium. Future studies will be needed to unravel the molecular mechanism

underlying destabilization of SAA hexamers by FABPs.

To explore how SAA dissociation initiates the release of IL-33 in airway epithelial cells, Smole and colleagues sought to determine which of the many known receptors for SAA were involved. Only interaction with the G-protein-coupled formyl peptide receptor 2 (FPR2) specifically resulted in robust epithelial IL-33 release. Conversely, the selective FPR2 inhibitor WRW4 significantly reduced both HDM-induced IL-33 expression in BEAS-2B cells in vitro and the development of allergic airway inflammation in vivo. Thus, it can be concluded that the FABP–SAA–FPR2 axis facilitates type II airway inflammation by releasing IL-33, which subsequently activates ILC2s and DCs (Fig. 1). Of note, activating the FABP–SAA–FPR2 axis is not the only mechanism by which allergens induce epithelial IL-33 release, since extracts of the *Alternaria alternata* mold do not have the capacity to dissociate SAA hexamers and trigger IL-33 release via protease activity and PAR-2 cleavage. On the other hand, Smole and colleagues found that lung inflammation following exposure to FABP-containing extracts from the *Schistosoma mansoni* parasite was reduced in *Saa*^{−/−} mice as compared to wild-type mice, indicating that activation of the FABP–SAA–FPR2 axis is not a unique feature of HDM allergens. However, an unanswered question that remains is how FPR2 engagement induces IL-33 release, as this requires changes in chromatin binding because of the unique nuclear localization of IL-33 in epithelial cells. Additional studies should also establish whether FPR2 triggers IL-33 release directly or indirectly, for example, via an autocrine loop involving IL-1 α ⁴. The identification of a critical role for FABPs in HDM-driven asthma suggests the need for further investigation into FABPs and their receptors and downstream signaling pathways in other allergic diseases, including allergic rhinitis, atopic dermatitis and food allergies.

The FABP–SAA–FPR2–IL-33 axis is certainly not the only pathway engaging SAA and FPR2 in the context of an allergic response. FPR2 is a promiscuous receptor that can be activated by a variety of host and pathogen-derived ligands, exerting opposing biological actions in many immune cells. Whereas binding of the proresolving lipid mediator lipoxin A4 (LXA4) reduces chemotaxis and the production of proinflammatory cytokines, binding of SAA promotes transmigration and the functional responses of neutrophils^{7,8}. It was reported that FPR2-deficient mice are protected from eosinophilic airway inflammation, which was associated with reduced immune cell

chemotaxis⁹. However, ovalbumin was used as an allergen, indicating that, in this model, either FPR2 is bound by another ligand, or SAA is induced by non-FABP molecules. IL-33 is not the only cytokine induced by the SAA–FPR2 interaction, as SAA can also trigger the release of IL-8, IL-10, GM-CSF and CCL2 by immune cells or airway epithelial cells via FPR2⁷. Moreover, SAA stimulates FPR2-mediated upregulation of matrix metalloproteinase 9, which is an important player in airway remodeling in asthma. It is conceivable that the outcome of allergen binding to SAA and the subsequent activation of FPR2 depend on an array of variables, including the composition of the allergen, the type of epithelial cells, the anatomical location within the lung and genetic factors. In this context, recent single-cell expression analyses revealed major gene expression differences between cells of the upper and lower airway and parenchyma, as well as altered communication between immune and structural cells in the lung in asthmatic inflammation¹⁰.

Finally, Smole and colleagues found support for altered SAA–FABPR-driven immune sensing in human disease. In nasal epithelial cells from patients with chronic rhinosinusitis (CRS) patients, and probably also in bronchial epithelial cells from asthmatics, expression of SAA and FPR2 was increased. Moreover, following HDM stimulation, epithelial cells from CRS patients exhibited increased SAA hexamer dissociation and IL-33 secretion as compared to control epithelial cells. In parallel, the lung epithelium of patients with chronic obstructive pulmonary disease (COPD) displays prominent FPR2 expression, and, in acute COPD exacerbations, SSA is elevated⁷. The finding by Smole and colleagues that local delivery of the FPR2 inhibitor WRW4 diminished eosinophilic lung inflammation in an HDM-induced asthma mouse model points to considerable therapeutic potential of FPR2 inhibition for asthma. Concomitantly, this elegant work highlights the need to expand our understanding of the

FABP–SAA–FPR2–IL-33 axis in allergic airway inflammation. 

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Competing interests

The author declares no competing interests.