

or other environmental exposures can have on the adaptive immune system.

The findings reported by Ito et al. (2016) also have translational implications. Indeed, while there has been a substantial amount of focus on the role of excess cholesterol on the cells typically associated with the innate immune system, particularly macrophages in the study of atherosclerosis, there is a relative paucity of understanding of this biology in the adaptive immune system. The findings in this current study should incite greater clinical interest in the relationship between cholesterol and autoimmune disease. Currently there is an ongoing discussion toward the use of the cholesterol-lowering statins for the purposes of treating autoimmune conditions such as rheumatoid arthritis, lupus, and others (Khattri and Zandman-Goddard, 2013). However, statins affect not only cholesterol synthesis but also the entire isoprenoid pathway, which is involved in fundamental functions including protein prenylation, N-linked protein glycosylation, and ubiquinone production. Further-

more, although statins have generally been assumed to have anti-inflammatory actions due to their effect in lowering systemic cholesterol, the effects of statins on protein prenylation have been shown to directly engage the inflammatory and promote the production of interleukin 1 β , thus promoting metaflammation and the onset of diabetes (Henriksbo et al., 2014; Henriksbo and Schertzer, 2015). The findings by Ito et al. (2016) suggest that pharmacological targeting of LXR could present an alternative to the use of statins for the treatment of autoimmune disease. More generally, these findings remind us that metabolic context has a major role in the onset and progression of a multitude of pathologies of an immunological nature.

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REFERENCES

- A-Gonzalez, N., Bensinger, S.J., Hong, C., Beceiro, S., Bradley, M.N., Zelcer, N., Deniz, J., Ramirez, C., Diaz, M., Gallardo, G., et al. (2009). *Immunity* 31, 245–258.
- Goldstein, J.L., DeBose-Boyd, R.A., and Brown, M.S. (2006). *Cell* 124, 35–46.
- Henriksbo, B.D., and Schertzer, J.D. (2015). *Adipocyte* 4, 232–238.
- Henriksbo, B.D., Lau, T.C., Cavallari, J.F., Denou, E., Chi, W., Lally, J.S., Crane, J.D., Duggan, B.M., Foley, K.P., Fullerton, M.D., et al. (2014). *Diabetes* 63, 3742–3747.
- Hotamisligil, G.S. (2006). *Nature* 444, 860–867.
- Ito, A., Hong, C., Oka, K., Salazar, J.V., Diehl, C., Witztum, J.L., Diaz, M., Castrillo, A., Bensinger, S.J., Chan, L., and Tontonoz, P. (2016). *Immunity* 45, this issue, 1311–1326.
- Khattri, S., and Zandman-Goddard, G. (2013). *Immunol. Res.* 56, 348–357.
- Tabas, I. (2002). *J. Clin. Invest.* 110, 583–590.
- Tall, A.R., and Yvan-Charvet, L. (2015). *Nat. Rev. Immunol.* 15, 104–116.

Allergen Exposure: When Timing Is Everything

Tanel Mahlaköiv¹ and David Artis^{1,*}

¹Jill Roberts Institute for Research in Inflammatory Bowel Disease, Joan and Sanford I. Weill Department of Medicine, Department of Microbiology and Immunology, Weill Cornell Medicine, Cornell University, New York, NY 10021, USA

*Correspondence: dartis@med.cornell.edu
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It is known that young children exposed to allergens are prone to develop asthma later in life. In this issue of *Immunity*, de Kleer et al. (2016) identify IL-33 as a key player in the developing lung for sensitization to environmental allergens and airway hyperreactivity.

Genetic and environmental factors are well known to influence the predisposition of children to develop allergic diseases. During the past few decades, the prevalence of childhood asthma has rapidly increased and now affects up to 5%–10% of the world's population. We have learned a great deal by studying patient samples and preclinical models of airway inflammation, which provide a comprehensive understanding of the genetic,

molecular, and cellular machinery that contribute to active disease. However, the factors that underlie the increased risk of asthma and allergy in children remain poorly understood.

Now, de Kleer et al. (2016) add a developmental element to the equation and provide a detailed characterization of the timeline of immunological events in the developing lung by using a mouse model of house dust mite (HDM)-induced respi-

ratory allergy. The postnatal phase of alveoli formation (alveolarization), which in humans lasts up to 3 years of age, is characterized by a rapid influx of immune cells involved in type 2 immune responses, such as group 2 innate lymphoid cells (ILC2s), eosinophils, and mast cells. It is noteworthy that during this early developmental period, these type 2 innate immune cell populations vastly outnumber their counterparts in adult mice, leading

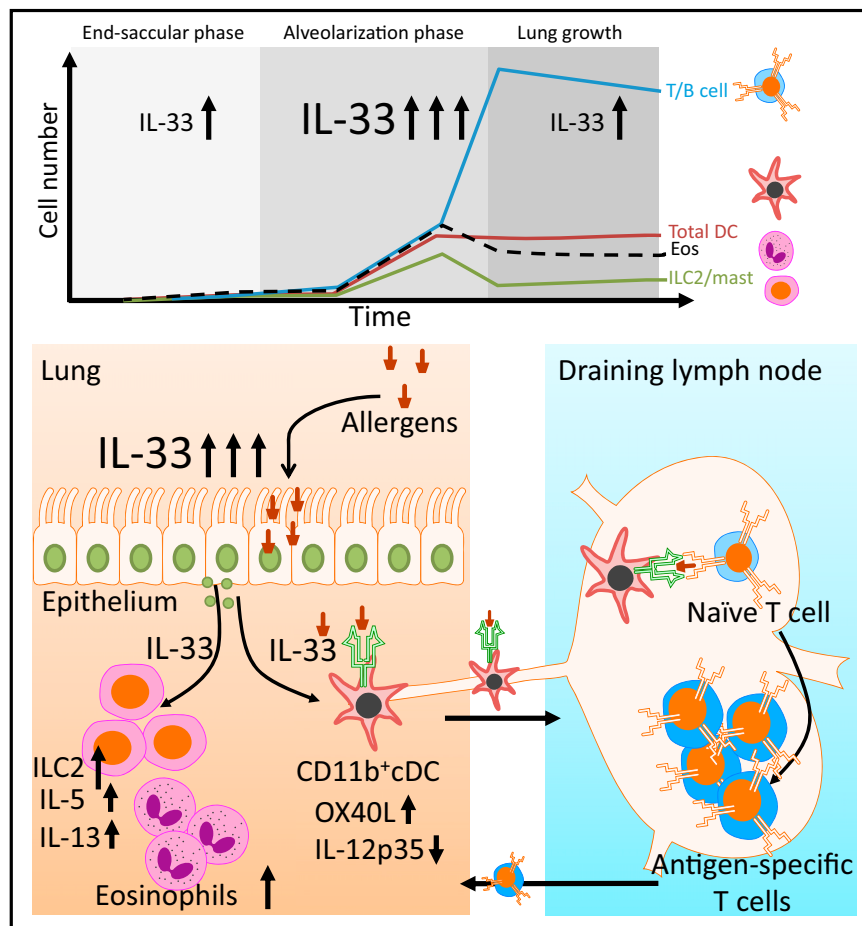


Figure 1. Perinatal Activation of the IL-33 Pathway Induces Type 2 Immunity and Allergen Sensitization in the Developing Lung

IL-33 is highly expressed during the alveolarization phase of lung development where it regulates ILC2, eosinophil, and mast cell recruitment to the lung. Dendritic cells, T cells, and B cells reach their peak numbers toward adulthood. HDM exposure during the alveolarization phase boosts IL-33 expression, ILC2 proliferation, and cytokine production. IL-33 induces OX40L and decreases IL-12p35 expression on CD11b⁺ DCs, and DC migration to lymph nodes, where DCs promote Th2 cell responses.

to a type 2 immune bias that is often associated with allergies. Cells of the adaptive immune system, T and B cells, and dendritic cells (DCs) are poorly represented during this postnatal period but gradually populate the lung and reach peak numbers once type 2 innate immune cell populations have dropped to post-developmental amounts (Figure 1). Importantly, exposure to HDM, specifically during this alveolarization phase, leads to an exaggerated innate immune response and effective sensitization of T helper 2 (Th2) cells to the allergen.

The pleiotropic cytokine interleukin-33 (IL-33) regulates type 2 immune cell numbers and their activity in homeostasis and inflammation. Recently, IL-33 has also been suggested to regulate lung

morphogenesis (Drake et al., 2016). de Kleer et al. (2016) demonstrate that high expression of IL-33 in the lung is developmentally regulated and its peak expression coincides with the influx of type 2 innate immune cells (Figure 1). Furthermore, ILC2 and eosinophil numbers directly depended on IL-33 signaling, which was reduced in mice lacking the IL-33 receptor (IL-33R). Considering that SNPs in human *IL33* and *IL1RL1* (IL-33R) genes have a strong association with asthma, it is tempting to speculate that IL-33 regulates the amount of Th2 cell immune skewing and the risk for becoming sensitized to environmental allergens in childhood (Moffatt et al., 2010).

Allergic airway hyperreactivity is the consequence of the activation of a wide

range of immune cells. Th2 cells are considered to be central in driving the disease by the production of type 2 cytokines IL-4, IL-5, and IL-13. Th2 cell activation results in eosinophilia, excessive mucus secretion, tissue damage, and tissue remodeling (Lambrecht and Hammad, 2015). However, after exposure to HDM, specifically during the alveolarization phase, a prominent type 2 immune response developed that was minimally dependent on Th2 cells (de Kleer et al., 2016). HDM exposure boosted IL-33 expression and strongly activated ILC2s. ILC2s are the innate counterparts of Th2 cells, sharing the expression of the transcription factor GATA-3 that drives the expression of type 2 cytokine genes. However, ILC2s lack antigen-specific receptors and are activated by epithelial-derived cytokines and alarmins, such as IL-33, thymic stromal lymphopoietin (TSLP), and IL-25 (Klose and Artis, 2016). Although ILC2s in the developing lung are outnumbered by T cells, it is the ILC2s and not naive T cells that mount early type 2 responses to epithelial-derived IL-33 (de Kleer et al., 2016). It has already been recognized that in some circumstances ILC2s contribute to allergic airway inflammation in a T-cell-independent manner (Lambrecht and Hammad, 2015). The predominance of ILC2s in causing a full-blown allergic response through production of IL-5 and IL-13 over Th2 cells during early lung development, however, is noteworthy.

Different allergens or infectious agents have different capacities to trigger the release of epithelial alarmins such as IL-33. In contrast to HDM exposure, neonatal rhinovirus induces IL-25-dependent ILC2 activation and IL-13 production, whereas IL-33 amounts remain constant (Hong et al., 2014). In the virus study, infection was performed in 6-day-old mice, coincident with the onset of the alveolarization phase and type 2 immune cell influx, which makes it difficult to directly compare with the HDM model. However, these models of childhood respiratory infection and allergy together suggest that ILC2s and ILC2-activating cytokines play an early role in lung development. Considering that amphiregulin (AREG), a member of the epidermal growth factor family that is elicited in ILC2s by IL-33, is involved in lung development, it remains to be seen

whether AREG-producing ILC2s could directly contribute to tissue development perinatally when T cells, a potential alternative source of AREG, are lacking (Schuger et al., 1996). Notably, it has been proposed that upon expansion, ILC2s are long lived and primed for rapid re-activation, therefore possessing “innate-like memory” (Martinez-Gonzalez et al., 2016). In addition, allergen exposure in immunodeficient mice lacking cells of the adaptive immune system and patients with eosinophilia, but without apparent Th2 cell response, demonstrate high levels of IL-5 and IL-13, suggesting T-cell-independent ILC2 activation (Lambrecht and Hammad, 2015). Whether the early population expansion of lung-resident ILC2s could have a long-lasting effect and may predispose an individual for T-cell-independent type 2 pathology later in life would be an interesting subject for a follow-up study. For example, rhinosinusitis patients are characterized by increased ILC2 numbers that correlate with worse symptom score and asthma; however, a causal relationship between increased ILC2 numbers and the disease has not been identified (Ho et al., 2015).

Respiratory allergens are picked up by DCs in the lung and presented to antigen-specific T cells in draining lymph nodes. Despite relatively low DC and T cell numbers in the lung during the alveolarization period, de Kleer et al. (2016) demonstrated strong T cell sensitization compared to pre- or post-alveolar phase. By dissecting the role of different DC subsets in allergen uptake and presentation, the authors identified that a small population of conventional CD11b⁺ DCs (cDCs) was most efficient in antigen uptake and CCR7 chemokine receptor-dependent migration to lymph nodes. ILC2-derived IL-13 has been shown to promote anti-

gen-presenting DC migration to lymph nodes and the induction of T cell response (Halim et al., 2014). Strikingly, in addition to IL-13, de Kleer et al. (2016) demonstrated yet another role for IL-33 during the early sensitization process: IL-33 signaling was crucial not only for CD11b⁺ cDC migration to lymph nodes but also for their Th2-cell-skewing capacity via promotion of OX40L and suppression of IL-12p35 expression, factors promoting Th2 and Th1 cell polarization, respectively. Thus, compared to their adult counterparts, neonatal DCs have a pro-Th2 cell “phenotype” that is largely promoted by IL-33R expression and signaling.

Lastly, the authors demonstrated that blocking IL-33 signaling by recombinant soluble IL-33R (rsIL-33R) during allergen sensitization suppressed most features of airway hyperreactivity upon challenge (de Kleer et al., 2016). IL-33 blocking was most effective in preventing sensitization if performed during the alveolarization phase and not earlier in development or later in adulthood. But can these data be translated to human disease? Mouse models of respiratory allergy do recapitulate some aspects of human disease in patients in terms of Th2-cell-dependent eosinophilia and pathology. However, given differences in lung physiology between humans and mice, coupled with proposed differences in the location and number of ILCs in human versus rodent airways, more patient-based studies will be required to address this important issue. Notwithstanding that, de Kleer et al. (2016) demonstrate that the IL-33 pathway is crucial for the development of the lung immune system. Remarkably, the ever-expanding list of pleiotropic functions of IL-33 upon allergen exposure now includes hyperactivating type

2 immune cells, stimulating DC migration and Th2 cell polarization, resulting in allergic inflammation and airway hyperactivity. These findings provide a framework to further delineate how myeloid, granulocytic, and lymphoid cell responses are influenced in distinct developmental windows and integrated to drive type 2 inflammation that may persist for prolonged periods. Most notably, in the immune system, as in business and life generally, timing is everything.

REFERENCES

- de Kleer, I.M., Kool, M., de Bruijn, M.J.W., Willart, M., van Moorleghe, J., Schuijs, M.J., Plantinga, M., Beyaert, R., Hams, E., Fallon, P.G., et al. (2016). *Immunity* 45, this issue, 1285–1298.
- Drake, L.Y., Squillace, D., Iijima, K., Kobayashi, T., Kephart, G., O'Brien, D., and Kita, H. (2016). *J. Immunol.* 196, 51.15.
- Halim, T.Y., Steer, C.A., Mathä, L., Gold, M.J., Martinez-Gonzalez, I., McNagny, K.M., McKenzie, A.N., and Takei, F. (2014). *Immunity* 40, 425–435.
- Ho, J., Bailey, M., Zaunders, J., Mrad, N., Sacks, R., Sewell, W., and Harvey, R.J. (2015). *Clin. Exp. Allergy* 45, 394–403.
- Hong, J.Y., Bentley, J.K., Chung, Y., Lei, J., Steenrod, J.M., Chen, Q., Sajjan, U.S., and Hershenson, M.B. (2014). *J. Allergy Clin. Immunol.* 134, 429–439.
- Klose, C.S.N., and Artis, D. (2016). *Nat. Immunol.* 17, 765–774.
- Lambrecht, B.N., and Hammad, H. (2015). *Nat. Immunol.* 16, 45–56.
- Martinez-Gonzalez, I., Mathä, L., Steer, C.A., Ghaedi, M., Poon, G.F.T., and Takei, F. (2016). *Immunity* 45, 198–208.
- Moffatt, M.F., Gut, I.G., Demenais, F., Strachan, D.P., Bouzigon, E., Heath, S., von Mutius, E., Farrall, M., Lathrop, M., and Cookson, W.O.C.M.; GABRIEL Consortium (2010). *N. Engl. J. Med.* 363, 1211–1221.
- Schuger, L., Johnson, G.R., Gilbride, K., Plowman, G.D., and Mandel, R. (1996). *Development* 122, 1759–1767.