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INSIGHTS

ILC2s chew the fat

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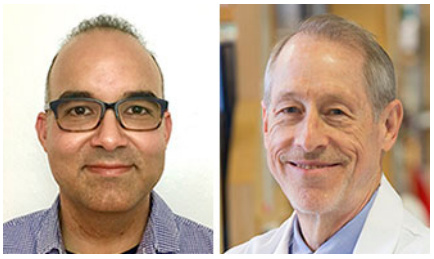
In this issue of *JEM*, Rana et al. (https://doi.org/10.1084/jem.20190689) report that adipose tissue multipotent stromal cells (MSCs) provide multifaceted support for adipose tissue–resident ILC2s through contact-mediated proliferation and IL-33–mediated stress-induced activation.

Allergy is a disease canonically associated with adaptive IgE-mediated aversive responses. The discovery of group 2 innate lymphoid cells, ILC2s, has provoked a new look at this arm of immunity and evokes the concept of "innate allergy," which remains confusing. In the mouse, ILC2s first appear in tissues in late fetal development and, upon birth, undergo massive proliferation and expansion while acquiring transcriptional programs attuned to the tissues in which they dwell (Schneider et al., 2019). As such, ILC2s resemble a spectrum of pre- and perinatally positioned tissue-resident immune cells—from fetal macrophages to γδ T cells to thymic T regulatory (T reg) cells—that hints at complex roles for the immune system in tissue homeostasis (Gasteiger and Rudensky, 2014). Indeed, ILC2s express a variety of cytokine, neuropeptide, and lipid receptors that have roles in integrating signals from diverse cell types in orchestrating innate allergy by their discharge of IL-5, IL-13, and other mediators.

Although developing after birth, white adipose tissue was noted to be infiltrated with ILC2s since their initial published description (Moro et al., 2010). Subsequent studies revealed ILC2s to be critical for sustaining healthy adipose tissue, in part by establishing immune tone necessary to curtail development of an inflammatory state and insulin intolerance (Molofsky et al., 2013). But why are ILC2s there? What keeps them there, and what do they do? Adipose ILC2s constitutively express high levels of the IL-33 receptor, ST2, and respond rapidly to exogenous IL-33 (Molofsky et al., 2015). In the current issue of *JEM*, Rana et al. used IL-33 reporter mice to identify the major reservoir of IL-33 to be adipose PDGFRα$^+$ multipotent stromal cells (MSCs), which the authors confirm by their capacity to differentiate into adipocytes or α-smooth muscle actin$^+$ myofibroblasts in vitro. Isolated adipose MSCs induced activation and proliferation of purified ILC2s in culture, but surprisingly, proliferation was maintained even by IL-33–deficient MSCs, though only when cell–cell contact was sustained by interactions between ICAM-1 on MSCs and LFA-1 on adipose ILC2s. In turn, cytokines from ILC2s induced eotaxin (CCL11), leading to tissue eosinophil influx. Thus, specialized adipose MSCs sustain constitutive ILC2s, since their numbers and activation profile were diminished in LFA-1–deficient mice, through both contact-mediated constitutive mechanisms and IL-33–mediated induced mechanisms, and ILC2 type 2 cytokines, likely IL-13, were required to maintain constitutive nuclear IL-33 levels in MSCs.

Interest in these adipose interactions has provoked contemporaneous investigations using independent approaches to track the relationship of ILC2s with tissue reservoirs of IL-33. Mahlakõiv et al. (2019) used IL-33 reporter mice to identify a similar population of adipose stromal cells, designated adipose stem and progenitor cells, as well as a lesser population of podoplanin$^+$PDGFRα$^-$ stromal cells in the fat pad–enveloping mesothelium. High-fat diet induced rapid IL-33 loss from adipose stem and progenitor cells and protection from the increases in weight and adipocyte numbers seen in the absence of IL-33 or ST2. The authors suggested that IL-33 from mesothelial lining cells might function more as an alarmin released after tissue perturbation through



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expansion or injury. Spallanzani et al. (2019), with a long interest in adipose ST2$^+$ T reg cells, also localized IL-33 predominantly to adipose PDGFRα$^+$Sca1$^+$ stromal and mesothelial cells, but single-cell RNA sequencing (RNA-seq) and bulk RNA-seq analysis parsed the former to define a more proliferative subtype with a prominent epithelial–mesenchymal transition profile. They suggested that "immunocyte-promoting" stromal cells constitute a unique adipose population, as compared with "adipocyte-generating" stromal cells by a process largely dependent on IL-33. Strikingly, adipose T reg cells and IL-33 were much more prominent in male as compared with female mice. Finally, Dahlgren et al. (2019) took an orthogonal approach by microscopically localizing ILC2s in several tissues to demonstrate their prevalence in adventitial cuffs, "virtual" collagen-rich matrices that surround intermediate blood vessels and provide conduits for collecting interstitial fluids for routing to lymphatics that egress from these specialized sites. ILC2s were also prominent in mesothelial serosa, including the pleura and adipose capsule as

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seen in the other studies. The adventitial stromal cells, or ASCs, supporting the ILC2 niches were PDGFRα⁺Sca1⁺podoplanin⁺ cells, the majority (~60%) of which expressed IL-33. Single-cell RNA-seq revealed expression of eotaxin (CCL11), but also thymic stromal lymphopoietin (TSLP), consistent with a tissue ILC2 niche, which was supported further by expression of IL-7 in lymphatic endothelia abutting the adventitial cuffs. Similar populations of ASCs were present in gonadal adipose tissue, as were the mesothelial IL-33⁺ capsular stromal cells, in agreement with the other studies. Activation of ILC2s drove expansion of IL-33⁺ lung ASCs which was mimicked by IL-13, and deletion of IL-33 from ASCs limited Th2 accumulation in the lung after migratory helminth infection.

Although there remain some discrepancies, each of these studies points to unique stromal cell populations as a major source of IL-33 at sites populated by ST2⁺ ILC2s (and likely T reg cells) and generates momentum for addressing the signals that lead to export of IL-33 protein from ASCs to target immune cells. ILC2s position normally in IL-33- and ST2-deficient mice, suggesting a role in activation and/or turnover rather than spatial organization by IL-33, but the ASCs have additional mechanisms (e.g., ICAM-1, TSLP) to impact ILC2s and other immune cells, as revealed by these studies. Are these specialized stromal cells related to the PDGFRα⁺podoplanin⁺ mesenchymal cells that mediate tissue differentiation of fetal ILC2 precursors in conjunction with Notch and IL-7 (Koga et al., 2018)? Do these stromal cells constitute the tissue "checkpoints" that release type 2 cytokines from infiltrating activated lymphocytes (Van Dyken et al., 2016)? The large datasets generated by these investigations will allow construction of more precise reagents to define subsets of these stromal populations that govern critical sites where immune cells leverage decisions between tissue support of homeostasis versus emergency interdiction of pathogenic invaders.

ILC2s (and T reg cells) are prevalent in cutaneous and mucosal tissues and are important for homeostasis in response to environmental perturbations mediated by microbiota, metabolites, food, and pathogenic insults. It is reasonable to ask whether the positioning of ILC2s and T reg cells in white adipose depots may be important in the integration of systemic metabolic demands as dictated by the peripheral tissues. Examples of such crosstalk have been noted. During *Trichuris muris* infection, inhibition of fatty acid metabolism led to a reduction in the number of ILC2s, their production of type 2 cytokines, and attenuation of worm expulsion that was not seen following inhibition of glucose utilization and was consistent with skewing of energy needs to fatty acids to generate the sustained mucosal response required to deal with this organism (Wilhelm et al., 2016). Conversely, infection with the migratory helminth *Nippostrongylus brasiliensis* improved insulin resistance otherwise associated with high-fat diet (Wu et al., 2011), consistent with systemically integrated metabolic alterations to meet specific tissue demands, but how these systemic responses become integrated in visceral adipose stores is unclear. Of note, *N. brasiliensis* infection was accompanied by mobilization of ILC2s into the circulation (Huang et al., 2018), thus creating a mechanism by which type 2 cytokines could be systemically disseminated, akin to hormones. In this way, the mobilization of resident ILC2s from tissue in response to local perturbations could distribute type 2 cytokines to adipose stores to induce levels of IL-33 on specialized stromal mesenchymal cells, as shown in the articles discussed above, and thus reset the metabolic tone of the tissue. Further study is needed to explore the interplay between ILC2 activation and IL-33–expressing stromal cells in adipose tissues, and what the metabolic consequences might be in parsing the line between host defense and tolerance in respect of tissue function (Ganeshan et al., 2019). The work by Rana et al. (2019), as well as those additionally highlighted, have begun to point the way forward in this fascinating area of biology addressing the evolutionary underpinnings of allergic immunity.

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