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Killer ILCs in the Fat

Emilie Narni-Mancinelli[1,*] and Eric Vivier[1,2]
[1]Centre d'Immunologie de Marseille-Luminy, Aix Marseille Université, INSERM, CNRS, 13009 Marseille, France
[2]Hôpital de la Timone, Assistance Publique-Hôpitaux de Marseille, 13009 Marseille, France
*Correspondence: narni@ciml.univ-mrs.fr
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Innate lymphoid cells (ILCs) residing in adipose tissue participate in the pathogenesis of obesity, but their contribution toward adipose tissue homeostasis in the lean state is unclear. Boulenouar et al. (2017) now report that heterogenous type 1 ILCs in adipose tissues regulate macrophage homeostasis through cytotoxicity.

Adipose tissue (AT) is central to energy storage and for the regulation of nutrition, systemic metabolism, and immunity. Immune cells are numerous in AT, their numbers being surpassed only by adipocytes. In the lean state, AT immune cells include T regulatory (Treg) cells, NKT cells, group 2 innate lymphoid cells (ILC2s), and M2 macrophages; these cell types have distinct roles in the maintenance of AT homeostasis (Yang et al., 2016). Obesity is associated with changes in the numbers, phenotype, and functions of AT immune cells. Numbers of resident Treg cells decrease, while the increased presence of pro-inflammatory cells fosters chronic inflammation and ultimately leads to insulin resistance (Yang et al., 2016). Recently, interferon (IFN)-γ produced by group 1 ILCs has been shown to participate in the pathogenesis of obesity by promoting macrophage differentiation to the inflammatory M1 phenotype (Lee et al., 2016; O'Sullivan et al., 2016; Wensveen et al., 2015). In this issue, Boulenouar et al. (2017) characterize the heterogeneity of type 1 ILCs in adipose tissues of humans and mice in lean and obese conditions. They find that the cytotoxic potential of these ILCs toward adipose tissue macrophages (ATMs) regulates adipose tissue homeostasis and impacts systemic metabolism.

AT-ILC characterization in obesity has been previously reported but no study has addressed their composition in the lean state. Boulenouar et al. (2017) first show that human visceral omental fat and murine epididymal white adipose tissue (WAT) are enriched in group 1 ILCs as compared to other tissues, with group 1 ILCs constituting the most abundant lymphocyte population in AT. Parabiosis experiments revealed that AT group 1 ILCs are tissue-resident cells, with only a minor contribution from the periphery, similarly to the well-defined liver-resident ILC1s. These cells do not constitute a homogeneous population. In the mouse, Ly49$^-$ AT1-ILCs could be subdivided into three subsets according to surface expression of CD49a and CD49b: double-negative immature NK cells (CD49a$^-$CD49b$^-$; iNK), a mixed ILC1-like (CD49a$^+$CD49b$^-$ and CD49a$^+$CD49b$^+$) population, and mature NK cells (CD49a$^-$CD49b$^+$; mNK) (Figure 1). The proportion of each of these subsets is unique to AT.

Previous studies defined mouse AT type 1 ILCs as c-kit$^-$CD127$^-$Tbet$^+$, showing also that these cells had no history of RORγt expression (Lee et al., 2016; O'Sullivan et al., 2016). While mNK cells and CD49a$^+$CD49b$^+$ mixed ILC1s are uniformly Eomesodermin$^+$ (Eomes) similar to splenic conventional NK cells, 15% and 60% of iNK cells and CD49a$^+$CD49b$^-$ ILC1s, respectively, are Eomes$^-$, highlighting a heterogeneous transcriptional programing. Further analysis showed the Tbet dependence of mNK cells and the Tbet independence of iNK cell and mixed ILC1 subsets. Finally, unlike liver ILC1s, AT1-ILCs do not express TRAIL and exhibit low levels of granzyme B. AT1-ILCs, however, are genetically labeled in IFN-γ reporter mice as other group 1 ILCs present in

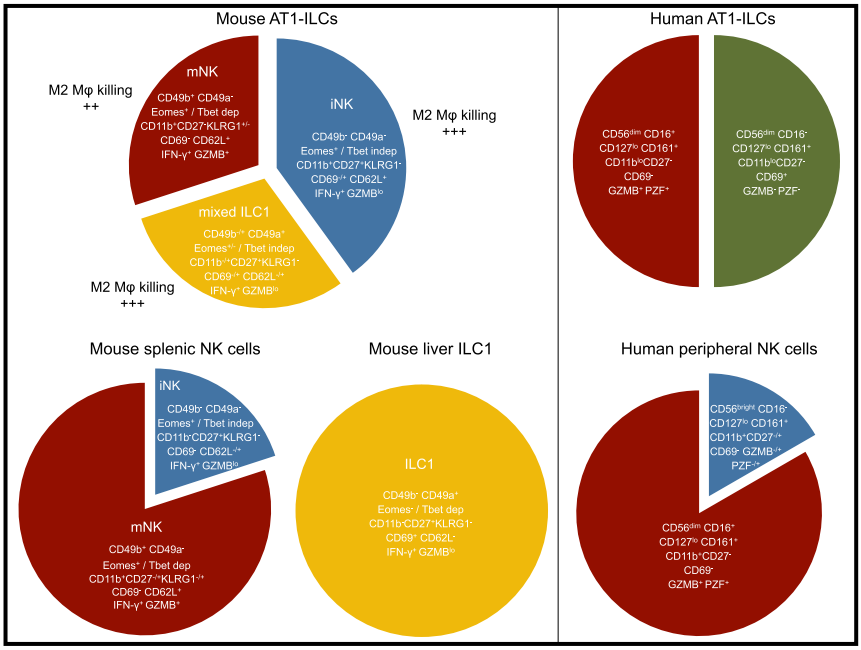

Figure 1. AT1-ILC Subsets in Mice and Humans

The heterogeneity of mouse and human AT type 1 ILCs is schematized with major phenotypic markers and transcription factors. Mouse AT1-ILCs can be subdivided in several subsets based on the expression of CD49b and CD49a integrins. iNK and mNK cells share common features with splenic NK cells whereas mixed ILC1s resemble liver ILC1s. Phenotype of splenic NK cells and liver ILC1s are indicated for comparison. Features of the human AT1 ILCs are shown with the enrichment in $CD56^{dim}CD16^{-}$ ILCs as compared to peripheral blood NK cells.

Abbreviations are as follows: AT1-ILCs, adipose tissue type 1 ILCs; iNK, immature NK cells; mNK, mature NK cells; dep, dependent; indep, independent; Eomes, eomesodermin; GZMB, granzyme b; PZF, perforin; IFN-γ, interferon gamma; Mφ, macrophage.

spleen or liver. Thus, mouse AT1-ILCs present unique features as compared to type 1 ILCs present in other tissues.

Human AT1-ILCs also exhibit a unique phenotype. Whereas blood NK cells are mostly composed of $CD56^{bright}CD16^{-}$ cells (5%) over $CD56^{dim}CD16^{+}$ (95%) cells, there are no $CD56^{bright}$ type 1 ILCs in human visceral omental. In addition, 50% of the $CD56^{dim}$ cells are $CD16^{-}$. Human AT is therefore enriched in an unconventional $CD56^{dim}CD16^{-}$ ILC1-like population with poor expression of cytotoxic molecules and also contains peripheral-like $CD56^{dim}CD16^{+}$ cells (Figure 1).

The number of peripheral NK cells is reduced in obese patients (Lynch et al., 2009). In concordance with these earlier findings, Boulenouar et al. (2017) find that omental AT in obese individuals contains a lower frequency of AT1-ILCs than that of lean counterparts. Interestingly, the numbers of AT1-ILCs increased in individuals who underwent bariatric surgery, indicating that restricted food intake can restore the numbers of AT1-ILCs to those seen in lean individuals. In mice, high-fat diet (HFD) feeding resulted in increased proliferation and recruitment of AT1-ILCs as early as 96 hr after the initiation of the HFD protocol, but prolonged HFD (12 weeks) led to a significant decrease in AT1-ILCs. As with bariatric surgery patients, weight loss was sufficient to restore the numbers of AT1-ILCs to the levels seen prior to HFD treatment. Thus, AT1-ILCs rapidly respond to changes in diet, in both human and mice, leading the authors to define them as "dynamic diet/energy sensors."

Obesity-induced inflammation is associated with aberrant adipose tissue macrophages (ATMs) M1 expansion and loss of AT1-ILCs. ATMs in lean mice express the stress-induced surface molecule Rae1, a ligand for the activating receptor NKG2D. Boulenouar et al. (2017) find that sorted AT1-ILCs could kill ATMs from lean mice in vitro, iNK cells and mixed ILC1s being surprisingly more effective killers than mNK cells. AT1-ILCs were more prone to kill M2 than M1 macrophages. In agreement with these data, further AT1-ILC depletion in vivo via the injection of anti-GM1 antibodies led to higher numbers of ATMs, of mostly the M2 phenotype. In perforin-deficient animals, the ratio of M2:M1 ATMs was higher than that in control mice, further suggesting that AT1-ILCs maintain ATM homeostasis through cytotoxicity, with preferential targeting of M2 ATMs. Obese mice have lower numbers of AT1-ILCs, and interestingly these cells displayed reduced cytotoxic activity as compared to AT1-ILCs from lean mice, suggesting that obesity impacts this AT1-ILC function. Transfer of AT1-ILCs from lean donor to obese mice improved glucose metabolism, suggesting that the reduced number and/or the impaired cytolytic function of AT1-ILCs contributes to glucose intolerance in obese mice. Taken together, these findings point to a regulatory role for AT1-ILCs in ATM homeostasis through cytotoxicity, with relevance both at steady state and in obesity.

The characterization of distinct AT1-ILCs subsets in human and murine AT and the revelation that the cytotoxic function of these cells has a role in AT homeostasis (Boulenouar et al., 2017) raises a number of interesting questions for future investigations. The signals responsible for AT1-ILC activation and regulation in the lean state remain to be defined; it is interesting to speculate that these may be AT-derived metabolites or cytokines. In chronic obesity, AT1-ILCs are decreased in numbers and intrinsically impaired in their killing ability against ATMs. This defect may be partly responsible for the increase in macrophages associated with obesity. Mouse AT1-ILCs are more effective at killing M2 rather than M1 macrophages. This result is puzzling since M2 macrophages are known to display beneficial effects on tissue homeostasis and anti-inflammatory properties. The authors hypothesize that M2 macrophages are indeed very active in lean adipose tissue engulfing and remodeling the AT. M2 macrophage elimination could thus prevent their harmful conversion into pathogenic pro-inflammatory M1-like macrophages. Furthermore, no metabolic disorders have been reported thus far in mice deficient for group 1 ILCs ($NKp46^{Cre}xROSA26^{flxDTAflx}$ mice) (Deauvieau et al., 2016) or in

humans or mice deficient in cytotoxicity (e.g., perforin deficiency) (Ménasché et al., 2005). It is also not obvious that patients devoid of ILCs (Vély et al., 2016) present abnormal AT distribution or metabolic disorders. The regulation of macrophage homeostasis and AT metabolism by ILCs remain to be explored in humans in natural conditions and undoubtedly represents an area of great interest.

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RSV Takes Control of Neonatal Breg Cells: Two Hands on the Wheel

Peter J.M. Openshaw[1,*]
[1]Respiratory Sciences, National Heart and Lung Institute, Imperial College London, St Mary's Campus, Norfolk Place, London W2 1PG, UK
*Correspondence: p.openshaw@imperial.ac.uk
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The viral attachment protein of RSV has many surprising features, especially its mimicry of fractalkine (CX3CL1). Zhivaki et al. (2017) now show that, in addition to using this homology to attach to ciliated cells, it activates human neonatal regulatory B cells, thereby inhibiting immunological responses.

Respiratory syncytial virus (RSV) is common pathogen that infects the ciliated respiratory epithelium, causing variable disease that ranges from severe infantile bronchiolitis to a mild (or unapparent) common cold. It infects virtually all children by the age of 3 and repeatedly re-infects them throughout life. It has two major surface proteins, one mediating fusion (F) and the other attachment (G). The latter has a structure similar to the chemokine fractalkine (CX3CL1), binding CX3CR1 on the apical surface of ciliated cells in the lung. In a new twist, Zhivaki et al. (2017) now show that RSV G also binds to a novel subset of neonatal regulatory B (nBreg) cells that make the immunosuppressive cytokine interleukin 10 (IL-10), thus inhibiting protective immune responses.

Rather than depending on rapid evolution to escape immune pressure, the success of RSV seems to depend on a partial but specific immunological amnesia (Openshaw et al., 2017). RSV's attachment protein G has few parallels in the viral kingdom. It is known to inhibit Toll-like receptor (TLR)-induced type I interferon host responses. One of the several interesting properties of RSV G is that vaccinating mice with it can induce enhanced and eosinophilic lung disease during subsequent RSV infection, a phenomenon that requires an intact central conserved domain (Sparer et al., 1998).

When the structure of CX3CL1 was first described in 1997, RSV aficionados immediately appreciated its close resemblance to RSV G. Both G and CX3CL1 have heparin binding domains, and both have membrane bound and soluble forms and both have "mucinoid" proximal regions on extended serine-threonine rich stalk that ends in a cysteine-rich chemokine domain (Melero et al., 2017). News of this remarkable similarity spread quickly in the RSV community and by 2001, Tripp et al. (2001) showed that the attachment protein G does indeed bind to the receptor CX3CR1. RSV exploits this property to infect the respiratory epithelium by binding of G to CX3CR1 on ciliated cells (Jeong et al., 2015; Johnson et al., 2015), although it remains a mystery why CX3CL1 should be present at this site.

CX3CL1 is chemoattractive for both lymphocytes and monocytes and is normally made by activated endothelial cells. Its receptor is naturally present on cells with high cytotoxic potential (such as natural killer [NK] cells, cytotoxic T cells, and γδ T cells). Presumably, the soluble form of RSV's attachment protein recruits