

INSIGHTS

Alveolar macrophages and epithelial cells: The art of living together

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In this issue of *JEM*, Gschwend et al. (2021. *J. Exp. Med.* <https://doi.org/10.1084/jem.20210745>) reveal the indispensable role of alveolar epithelial cells type 2 in controlling the density of alveolar macrophages. This study highlights the intricate crosstalk that lung stroma and macrophages undergo to maintain homeostasis.

Beyond their immune scavenging functions, macrophages perform critical tissue-specific roles to maintain homeostasis. In the lung, alveolar macrophages (AMs) are essential for the catabolism of surfactants generated by alveolar epithelial cells type 2 (AT2s). Consequently, absence or malfunction of AMs results in the development of a disease called pulmonary alveolar proteinosis in human and mice (Trapnell et al., 2019). What are the factors that control the density and function of tissue-resident macrophages? Despite being described centuries ago, we are just now unraveling the true cellular pathways and tissue trophic factors that control each population of tissue-resident macrophages. Work by Gschwend et al. in this issue of *JEM* brings light to one of these pathways: the development and maintenance of AMs through AT2-derived GM-CSF (Gschwend et al., 2021).

It is well established that AM density depends on GM-CSF as shown in *Csf2*-deficient mice (Guilliams et al., 2013). GM-CSF acts together with TGF β to instruct the differentiation of lung fetal monocytes to AMs through the activation of the transcription factor peroxisome proliferator-activated receptor γ (PPAR γ ; Yu et al., 2017; Schneider et al., 2014). The sources of lung GM-CSF at steady state include immune and nonimmune cells, i.e., basophils, type 2 innate lymphocytes (ILC2s), and epithelial cells (especially AT2s; Cohen et al., 2018;

Guilliams et al., 2013; Sheih et al., 2017; Schneider et al., 2014). However, the capacity of GM-CSF derived from immune versus nonimmune cells to modulate AM density during development and adulthood is still unclear.

To address this question, Gschwend et al. (2021) developed a new reporter and conditional knockout (cKO) mouse model that allows tracking GM-CSF-expressing cells and depleting this trophic factor in a cell-specific manner (*Csf2*^{flox-tdTomato}; *Csf2*^{fl}). First, the authors systematically mapped the cellular source of GM-CSF in the neonatal and adult lung and confirmed that this trophic factor is produced by immune and nonimmune cells. Within neonatal immune cells, ILC2s produced the highest levels of GM-CSF, followed by $\gamma\delta$ T cells. ILC2 capacity to produce GM-CSF agrees with a previous report (Cohen et al., 2018), and was maintained after birth in the adult lung. Of note, ILC2s produced higher levels of GM-CSF on a per-cell basis compared to nonimmune cells, as shown by the mean fluorescent intensity of tdTomato and confirmed by quantitative RT-PCR.

The high levels of ILC2-derived GM-CSF suggest that these cells may be implicated in the development and maintenance of AMs. To investigate that, the authors deleted GM-CSF from all immune cells by crossing the newly generated *Csf2*^{fl} mice to *Vav1*^{iCre} mice (*Csf2*^{Vav1} cKO). Intriguingly, *Csf2*^{Vav1} cKO



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mice harbored normal numbers of AMs in the lung. To further corroborate these findings, the authors show that depletion of lymphocytes or basophils had no effect on AM density in the neonatal and adult lung. Altogether, these results demonstrate unequivocally that GM-CSF derived from immune cells is dispensable for AM development and maintenance.

Next, Gschwend et al. (2021) analyzed the role of GM-CSF derived from non-immune cells on AM density. Beautiful microscopy detected high numbers of nonimmune cells producing GM-CSF, and flow cytometry profiling confirmed that these cells are indeed pro-surfactant protein C (SPC)-positive AT2s, as previously observed (Cohen et al., 2018; Sheih et al., 2017). By crossing *Csf2*^{fl} mice with *SPC*^{Cre} mice (*Csf2*^{SPC} cKO), GM-CSF was precisely depleted from AT2s, which reduced the total amount of lung GM-CSF by at least half.

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Importantly, *Csf2*^{SPC} cKO neonatal and adult mice had a complete depletion of AMs, unquestionably demonstrating the indispensable role of AT2-derived GM-CSF in AM development. To gain mechanistic insight, the authors showed that *Csf2*^{SPC} cKO mice developed signs of pulmonary alveolar proteinosis, i.e., accumulation of cellular debris, proteins, and cholesterol in the bronchoalveolar space (Gschwend et al., 2021). Finally, to evaluate the role of AT2-derived GM-CSF in adult AM maintenance, Gschwend et al. crossed *Csf2*^{fl} mice to tamoxifen-inducible *SPC*^{CreERT2} mice. Temporal depletion of AT2-derived GM-CSF resulted in the elimination of AMs in the adult lung, demonstrating that AT2s play an indispensable role not only in the development, but also in the maintenance of AMs.

The work from Gschwend et al. (2021) in this issue of *JEM* highlights the mutually beneficial symbiotic relationship that exists between AT2s and AMs. AT2-derived GM-CSF is indispensable for AM development and maintenance (Gschwend et al., 2021). AT2-derived GM-CSF instructs AM identity by promoting PPAR γ expression (Schneider et al., 2014; Yu et al., 2017). In turn, mature AMs are critical to eliminate AT2-produced surfactants and maintain a healthy lung environment. AT2s start shaping the lung microenvironment early in development between embryonic day 16.5 and 18.5 by timely producing GM-CSF, which in turn promotes the in situ proliferation of lung fetal monocytes and their differentiation to AMs (Gschwend et al., 2021; Guillems et al., 2013). In sum, AT2s build a nurturing lung microenvironment for AMs in exchange for macrophage scavenging functions (Guillems et al., 2020): a great example of mutualistic symbiosis at a cellular level.

The approaches used by the authors allowed them to precisely identify and eliminate the cellular sources of GM-CSF. Alternative approaches to shed light on cell-cell interactions are high-dimensional single-cell technologies. By applying single-cell RNA sequencing, the group of Dr. Amit identified another cellular partner of AMs, i.e., basophils (Cohen et al., 2018). Although basophils are dispensable for AM development (Gschwend et al., 2021), they promote the anti-inflammatory function of AMs at steady state (Cohen et al., 2018). Interestingly, basophils function in the lung is imprinted by GM-CSF and IL-33. Future work

should aim to identify the cellular source of GM-CSF that promotes the lung-specific signature of basophils.

Another area in need of further study is the mechanism by which AT2-derived GM-CSF regulates AM density. Even though AT2s are numerous in the lung, ILC2s seem to produce a greater amount of GM-CSF on a per-cell basis (Cohen et al., 2018; Gschwend et al., 2021); however, ILC2-derived GM-CSF is dispensable even when AT2s are unable to secrete GM-CSF. Why? This puzzle could be answered using imaging technologies aiming to recognize the local interaction between AT2s, AMs, and ILC2s. Also, it would be of interest to discern in which circumstances, if any, ILC2-derived GM-CSF plays an indispensable role in the lung microenvironment. Dissecting tissue-specific cell-cell crosstalk during homeostasis would help shed light on dysfunctional interactions occurring in disease states.

Although several mouse models exist for macrophage depletion, we still lack models that eliminate tissue-specific populations. The tools generated by Gschwend et al. (2021) support a means to delete precisely and temporarily AMs by taking advantage of the nonredundant function of AT2-derived GM-CSF. These tools have the potential to unravel novel AM biology in health and disease. Future avenues of research should aim to generate new mouse models that take advantage of macrophage-stromal cell interactions. We foresee that dissecting the intricate crosstalk between tumor-associated macrophages and stromal cells would result in mouse models for macrophage depletion, which will provide a unique opportunity for scientific discovery in the myeloid immunotherapy field.

Besides being involved in AM density and function, GM-CSF is emerging as a central player in the pathogenesis of multiple chronic inflammatory diseases (Hirota et al., 2018; Komuczki et al., 2019; Reynolds et al., 2016). Consequently, antibodies targeting GM-CSF have been tested clinically generating promising preliminary results (Hamilton, 2020). GM-CSF acts on infiltrating myeloid cells; however, the source of this trophic factor is disease-specific and, unfortunately, has only been partially mapped in most cases. Consequently, reporter and cKO mice such as the one described in the manuscript of Gschwend et al. (2021) provide a unique opportunity to pinpoint the source GM-CSF

and unravel the immune mechanisms that result in the pathogenesis of the disease. As an example, a published study from the group of Dr. Becher used a fate-mapping and reporter system to track GM-CSF-producing pathogenic T cells during experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis (Komuczki et al., 2019). This system allowed the authors to recognize a distinct and stable population of pathogenic T cells, ultimately revealing an important therapeutic target.

GM-CSF also has a vital role during acute viral infections, although its effects seem to be pleiotropic. On one hand, it plays a detrimental role by inducing excessive inflammation (Hamilton, 2020). On the other hand, it can play a beneficial role by promoting myeloid cell-mediated tissue repair and initiation of adaptive immune responses (Halstead et al., 2018; Unkel et al., 2012). With this in mind, administration or inhibition of GM-CSF is being tested therapeutically in COVID-19 patients (Lang et al., 2020). The cellular sources of GM-CSF during viral infections are unknown and require further investigation. However, the fact that GM-CSF can be derived from several sources and has pleiotropic effects raises several questions: (i) Does the nature, site, and stage of viral infection determine the cellular source of GM-CSF? (ii) Does the cellular source of GM-CSF determine the outcome of the immune response? (iii) Are the mechanisms of GM-CSF secretion cell and tissue specific? Genetically engineered mouse models are extremely valuable to provide insight into the pathways behind infections, especially models that allow controlling GM-CSF expression in a temporal manner. In short, we expect the scientific community will readily adopt the GM-CSF mouse models developed by Gschwend et al. (2021), which will result in new insights on the GM-CSF-dependent mechanisms regulating myeloid cell tissue density and function in health and disease.

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