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Research paper

Alveolar Macrophages

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ABSTRACT

Alveolar macrophages are the most abundant innate immune cells in the distal lung parenchyma, located on the luminal surface of the alveolar space. They are the first to encounter incoming pathogens and pollutants and to help orchestrate the initiation and resolution of the immune response in the lung. Similar to other tissue-resident macrophages, alveolar macrophages also perform non-immune, tissue-specific, homeostatic functions, most notably clearance of surfactant. In this review we will discuss how ontogeny and local lung environment shape the role of alveolar macrophages in health and disease.

1. Introduction

Alveolar macrophages are the most abundant innate immune cells in the distal lung, located on the luminal surface of the alveolar space. They are the first to encounter incoming pathogens and pollutants and help orchestrate the initiation and resolution of the immune response in the lung. Similar to other tissue-resident macrophages, alveolar macrophages also perform non-immune, tissue-specific, homeostatic functions, most notably clearance of surfactant. The immune and homeostatic functions of alveolar macrophages have been recently reviewed [1–4]. Considerable debate has surrounded the origin of these cells: while some studies indicated that alveolar macrophages were capable of proliferation, others suggested that circulating monocytes help maintain the alveolar macrophage pool. Recent scientific and technical advances have substantially improved our understanding of alveolar macrophage ontogeny. Here we will discuss how ontogeny and the lung microenvironment shape the role of alveolar macrophages in health and disease.

2. Identification and tracking of alveolar macrophages in the laboratory

Exposure to high partial oxygen pressure, surfactant and signals provided by alveolar type I and II cells produce a distinct alveolar macrophage phenotype which can be identified using multiparameter flow cytometry. The phenotype of mouse alveolar macrophages is well-described: similar to macrophages in other tissues they express MerTK, CD64, CD68, CD206 and F4/80. However, because of their adaptation to the unique lung environment, steady state mouse alveolar macrophages do not express fractalkine receptor CX3CR1 or integrin CD11b [5–8]. Instead, they express high levels of integrin CD11c, and sialic acid-binding lectin Siglec F [5–12]. These markers allow separation of true long-living alveolar macrophages (i.e. cells that have adapted to the local microenvironment) from transient monocyte-derived cells that were recruited to the alveolar space during injury [7,10,13–16]. Normal human alveolar macrophages express CD206, CD169, CD11c, CD163 and MARCO [17–21] which are also expressed by alveolar macrophages in non-human primates [22]. Alveolar macrophages are known to be highly autofluorescent. Although high autofluorescence may complicate flow cytometric analysis of alveolar macrophages, making them to appear falsely positive for a given marker, this feature can aid in their identification [23–25].

Numerous genetic systems exist for tracking and targeting macrophages, including alveolar macrophages. These include: simple transgene systems [26–28], Cre/lox systems (in particular *Cx3cr1, LysM, Cd11c, Runx1*) [13,14,26,28,29], tetracycline-inducible Tet-on systems [16], bone marrow chimeras including chimeras with thoracic shielding [5,12,30–32], adoptive transfer of macrophages and their precursors [8,33,34], and parabiosis and depletion of alveolar macrophages [5,12]. Use of Cre/lox-based systems for targeting macrophages and tools for depletion of macrophages, including alveolar macrophages, has been extensively discussed [35–37]. Importantly, none of these systems are specific for alveolar macrophages and often target other cell types, particularly other myeloid cells (such as monocytes and dendritic cells) and in some cases non-immune cells, for example, the Cre^{LysM} system, targets myeloid cells in addition to alveolar type II cells [38].

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3. Ontogeny and maintenance of alveolar macrophages in the steady state

The origin of alveolar macrophages has long been debated. Studies performed by van Furth over a century ago suggested that circulating monocytes give rise to all tissue-resident macrophages, including alveolar macrophages at steady state [39,40]. This concept has been revised during the past decade. Multiple studies have unambiguously demonstrated that some prototypical tissue-resident macrophages, such as microglia in the brain, Kupffer cells in the liver and large peritoneal macrophages in the peritoneal cavity, originate from various progenitors, populate tissues during different stages of embryogenesis and maintain their population throughout their lifetime with minimal to no contribution from circulating monocytes [35,41]. Although several waves of macrophages populate the lung during embryogenesis and participate in organogenesis, these do not give rise to the definitive alveolar macrophage population, since the alveolar niche does not exist until birth [8,11,28,42]. Thus, the first breath of a newborn may be viewed as the lung's first "wound": it creates a niche, which gets rapidly populated by circulating fetal monocytes.

The differentiation of recruited monocytes into alveolar macrophages and subsequent engraftment in the alveolar niche depends on the production of granulocyte-macrophage colony-stimulating factor (GM-CSF) by alveolar type II cells [8,11]. Loss of GM-CSF signaling, due to genetic mutation or development of autoantibodies against GM-CSF or its receptor, results in the loss of mature alveolar macrophages, accumulation of surfactant and the development of alveolar proteinosis [43]. Rescue of GM-CSF signaling by adoptive transfer of alveolar macrophages or their precursors with functional GM-CSF receptor or administration of exogenous GM-CSF can reverse this condition in mouse models [8,44–48]. In addition, there are reports of successful resolution of alveolar proteinosis after bone marrow transplantation in humans [49–51]. In contrast, M-CSF signaling is dispensable for survival of mature alveolar macrophages [52]. GM-CSF signaling is required for the expression of peroxisome proliferator-activated receptor gamma (PPARG) – a key transcription factor necessary for realization of the transcriptional program driving macrophage adaptation to the lung environment and acquisition of a high lipid load [6,8,53–55]. PPARG deletion in myeloid lineage prevented formation of mature alveolar macrophages, which resulted in delayed bacterial clearance after infection with *S. pneumoniae* and resulted in alveolar proteinosis. Other transcriptional factors, such as Bach2 and C/EBPβ are also required for development and maintenance of alveolar macrophages [56,57]. L-plastin is another protein that is required for the transmigration and retention of alveolar macrophage precursors into the alveolar niche: mice deficient for the *Lcp1* gene in myeloid cells fail to develop functional alveolar macrophages and have impaired clearance of pulmonary pathogens [42]. Recently, a crucial role of autocrine TGFβ signaling in development and maintenance of alveolar macrophages has been also demonstrated [58].

Using various fate-mapping techniques, several groups have demonstrated that in naïve, unchallenged adult mice housed in clean facilities, alveolar macrophages, microglia in the brain, Kupffer cells in the liver and large peritoneal macrophages maintain their population via proliferation *in situ* for months without contribution from circulating monocytes [5,8,14,26,29,32]. Supporting these observations made in mice, two recent clinical reports demonstrated that following lung transplant, the population of alveolar macrophages is remarkably stable with donor cells comprising the bulk of the alveolar macrophage pool even 5 years after transplant [59,60]. However, it is not known whether the initial seeding of alveolar macrophages into the lung during the postnatal period occurs as a single wave with subsequent expansion via local proliferation, or if additional alveolar macrophages derived from adult bone marrow-derived monocytes contribute to the alveolar macrophage pool during the rapid period of physiological body growth and lung niche expansion. Since alveolar macrophages are relatively sessile cells, [27,61] it is possible that during the period of active postnatal lung growth, alveolar macrophages that populated the lung soon after birth cannot migrate into newly formed alveoli. Hence, recruitment of monocytes from the circulation may be necessary in order to fully populate the niche [62]. Supporting this hypothesis, Gomez-Pedriguero and colleagues, using a *Flt3*-Cre reporter system which labels all cells derived from definitive hematopoiesis, found that at 4 weeks of age, 10–20% of alveolar macrophages were positive for the fluorescent reporter and that the proportion of these cells increased up to 40% by one year of life [63]. Perhaps stereological techniques combined with new "Microfetti" fate mapping models [64] which can track the clonal expansion of individual macrophages, might help further address the question of alveolar macrophage ontogeny and maintenance during physiological lung growth.

While it is certain that alveolar macrophages are long-lived, current fate mapping studies do not cover the entire lifespan of the animal. It was recently demonstrated that normal unperturbed aging is associated with decreased number of alveolar macrophages and downregulation of genes involved in cell cycle pathways [65]. Thus, it is possible that in the context of aging, increased environmental stress (such as exposure to airborne particulate matter over the long period of time) may lead to the recruitment of monocyte-derived alveolar macrophages in adults.

This raises the question: does the origin of alveolar macrophages matter? Several investigators have leveraged powerful genomic tools to help find an answer. Building on work from the ImmGen consortium [6], Lavin and colleagues demonstrated that the transcriptome and epigenetic landscape of tissue macrophages are determined by the tissue-specific microenvironment [66]. Using bone marrow transfer into whole body-irradiated hosts, they found that the lung microenvironment shapes the epigenetic landscape of bone marrow-derived alveolar macrophages, inducing a convergence with the epigenetic landscape of tissue-resident alveolar macrophages from naïve mice. Moreover, upon adoptive transfer into the alveolar space, mature peritoneal macrophages adapt to the niche, rapidly reshape their transcriptome and become somewhat alveolar macrophage-like cells. In another study, Gibbings and colleagues used congenic (CD45.1/CD45.2) bone marrow chimeras with thoracic shielding to protect the recipient's tissue-resident alveolar macrophages (a system originally introduced by Janssen and colleagues [32]), followed by partial depletion of these cells using clodronate-loaded liposomes, to generate a mouse that harbors alveolar macrophages of embryonic and adult origin simultaneously [12]. Upon opening the niche, monocytes entered the uninjured alveolar space and differentiated into cells which were virtually indistinguishable from tissue-resident alveolar macrophages both immunophenotypically and functionally. They further isolated tissue-resident and monocyte-derived alveolar macrophages via FAC-Sorting and subjected them to transcriptomic profiling via microarray. Only 35 probes were differentially expressed between tissue-resident and monocyte-derived cells. Similarly, van de Laar and colleagues demonstrated that upon adoptive transfer into a permissive niche, yolk sac macrophages, fetal monocytes and adult bone marrow monocytes all gave rise to cells that were functionally and transcriptionally indistinguishable from tissue-resident alveolar macrophages derived from fetal monocytes [33]. Together, these studies clearly demonstrate that in the unperturbed lung, at steady state, the origin of alveolar macrophages does not matter, as cells of fetal and adult origin as well as differentiated alveolar macrophages can occupy the permissive lung niche and give rise to fully functional, long-living, self-maintaining alveolar macrophages, which are virtually identical at the phenotypic, functional, genomic and epigenomic levels.

4. Origin and function of alveolar macrophages during aging, stress and lung injury

The aforementioned studies show that lung environment, and not cell ontogeny (embryonic versus adult), is a major driver of alveolar