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Series: Tissue-Resident Immune Cells

Review

Development and Function of Arterial and Cardiac Macrophages

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Macrophages inhabit all major organs, and are capable of adapting their functions to meet the needs of their home tissues. The recent recognition that tissue macrophages derive from different sources, coupled with the notion that environmental cues and inflammatory stimuli can sculpt and agitate homeostasis, provides a frame of reference from which we can decipher the breadth and depth of macrophage activity. Here we discuss macrophages residing in the cardiovascular system, focusing particularly on their development and function in steady state and disease. Central to our discussion is the tension between macrophage ontogeny as a determinant of macrophage function, and the idea that tissues condition macrophage activities and supplant the influence of macrophage origins in favor of environmental demands.

Introduction

The past decade has revolutionized our understanding of macrophage development by challenging a half-century-old idea that tissue macrophages are primarily monocyte-derived. We now know that, in the steady-state, macrophages in tissues such as the brain, liver, spleen, lung, peritoneum, gut, aorta, and heart have inhabited those tissues since embryogenesis with, in some cases, only minimal dependence on monocytes [1,2]. In response to infection or injury, however, monocytes infiltrate tissues in large numbers and give rise to macrophages that can be somewhat difficult to distinguish from their tissue-resident counterparts.

These insights, as significant as they are, also raise important questions. Does ontogeny dictate function? What are the functions of tissue-resident macrophages beyond those classically described? Are tissue-derived and monocyte-derived macrophages distinct, and if so how? Can tissue-derived macrophages proliferate throughout life, or do they rely on still-unknown progenitors? Here we consider these questions in the context of the current understanding of the ontogeny and function of macrophages residing in the heart and vessel walls, the two locations most dramatically affected by atherosclerosis and myocardial infarction. Because these diseases claim more lives than any others worldwide [3] understanding the role of macrophages therein may have broad clinical benefits.

Tissue Macrophages

In 1984 van Furth and Diesselhoff-den Dulk reported on the dual origins of splenic macrophages [4]. Through a series of experiments designed to profile proliferation and cell origin, the investigators concluded that approximately half of all splenic macrophages derive from monocyte

Trends

Cardiac and arterial macrophages first seed their respective tissues during embryogenesis prior to the establishment of definitive hematopoiesis.

The brain is seeded by yolk sac macrophages that remain in the organ into adulthood. In other tissues, subsequent waves of colonization occur that rely on fetal liver monocytes and, after birth, bone marrow-derived monocytes. The relative contributions of tissue-derived versus monocyte-derived macrophages are organ-specific and may be age-dependent.

In the steady-state adult aorta, macrophages reside predominantly in the adventitia. These macrophages derive from a brief post-birth monocyte colonization that replaces an earlier embryonic pool.

Macrophages reside throughout the heart in steady state and are interspersed between cardiomyocytes. Most cardiac macrophages are embryonically derived, although it has been argued that monocyte-derived macrophages colonize the organ and replace tissue-resident cells progressively throughout life.

In myocardial infarction and atherosclerosis, bone marrow- and spleen-derived monocytes infiltrate the myocardium and intima and give rise to macrophages. It is unknown whether these cells integrate with the resident pool.

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influx whereas the remainder depend on local macrophage production through self-renewal. At a time when macrophages were thought to be exclusively monocyte-derived, as formulated by van Furth and Cohn 16 years earlier [5], the idea that macrophages self-renew without monocytes did not easily catch on; to this day, the 1968 paper has nearly 10-fold more citations than its 1984 counterpoint. Over the years, numerous reports implicated local macrophage proliferation as a possible mechanism by which macrophages sustain themselves in the peritoneum [6,7], lungs [6,7], brain [8,9], skin [10], and aorta [11–16], but the broader implications of these early findings have only recently been explored. In many cases, the early studies relied on immunostaining strategies where cells co-expressing a myeloid or macrophage marker (i.e., CD68, F4/80) with a cell-cycle marker (Ki67, PCNA) were interpreted as evidence of proliferating macrophages. However, this strategy is problematic because of lack of specificity and uncertainty as to magnitude of phenomenon. CD68, for example, is not specific to macrophages [17], and a few cells expressing cell cycle markers – which signify that a cell is, or has been, in a particular stage of mitosis – tell us little about the importance of the process to the population as a whole. While surgical procedures such as parabiosis, where mice are joined together so as to share a common circulation, can inform about the influence of circulating cells on the replenishment of tissue-resident leukocytes, they do not address ontogeny.

If macrophages can either derive from monocytes or from local proliferation, does it follow that macrophages are ontogenically heterogeneous? Contemporary cell fate-mapping approaches show that macrophages seed the skin [18], brain [19], and many other tissues [20,21] during embryogenesis, before the emergence of monocytes. The studies typically rely on lineage tracing. One powerful strategy takes advantage of the fact that embryonic macrophages express the fractalkine receptor CX3CR1 (chemokine CX3C motif receptor 1), which can be used as a tag. Mice engineered with tamoxifen-inducible Cre recombinase (Cre fused to estrogen receptor fragment ERT2) under the direction of the *Cx3cr1* promoter are bred with mice containing the *Rosa26* locus into which has been inserted stop codon flanked by *loxP* sequences upstream of a gene coding for a fluorochrome such as enhanced yellow fluorescent protein (EYFP) [20]. In the absence of any intervention, the stop codon prevents fluorochrome expression. However, tamoxifen injected to pregnant mice drives nuclear translocation of the Cre–ERT2 fusion protein in CX3CR1-expressing cells of the pups (i.e., primitive macrophages). This leads to excision of the stop codon by Cre recombinase, permanently marking all $CX3CR1^{+}$ cells and their progeny with EYFP. If tamoxifen is injected before the emergence of definitive hematopoiesis, monocytes remain $EYFP^{-}$. The strategy, therefore, can distinguish cells that arise independently of hematopoietic stem cells from those that arise earlier in embryogenesis [20]. It is worthwhile mentioning, however, that the strategy, as powerful as it may be, is limited to cells expressing CX3CR1, and can confound results if the Cre–ERT2 fusion protein is either leaky or weakly translocated after tamoxifen injection. Thus, this particular strategy precludes conclusions regarding potential $CX3CR1^{-}$ progenitors that may be vital to macrophage biology and, indeed, alternative lineage-tracing approaches have led to some conflicting results on the nature of macrophage development [22].

That said, by using the CX3CR1 system we have learned that the yolk sac (YS) is the first source of macrophages during development, and thus constitutes the first macrophage colonization wave, which occurs around embryonic day 8.5 (E8.5) [23,24]. A second wave occurs when fetal liver monocytes, which ultimately derive from YS-derived erythro-myeloid progenitors (EMP) [25], and which are produced at E13.5, settle most organs and replace the first wave of YS-derived macrophages [26]. At birth, when hematopoiesis shifts to the bone marrow, macrophage colonization is complete in some locations but incomplete in others. The brain requires no further monocyte input while, in the intestine, monocytes continuously replace resident macrophages [27]. Thus, in most organs with the exception of the brain, at least three macrophage colonization waves occur: YS-derived macrophages appear in the first wave; fetal liver