Breaking the macrophage code in atherosclerosis

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Modelling human atherosclerosis is challenging. Current murine models of atherosclerosis are monofactorial hyperlipidemia-based models that are considered by cardiologists and vascular biologists as poor substitutes of human atherosclerotic disease due to disease site differences, lack of reproducible plaque rupture in a reasonable time frame, and different lipoprotein status[1]. Larger animal models are deemed more translationally and anatomically relevant to humans, yet our knowledge of their immune system is not fully serviceable. The immune system plays an important role in cardio-vascular health and disease[1] and we cannot afford the luxury of not taking into account its role in cardiovascular disease for optimal therapeutic targeting. Murine models are here to stay to model the role of immunity in atherosclerosis.

In this issue of *Cardiovascular Research*, Zernecke and colleagues[2] use new single cell biology tricks to try answer the age-old question of how murine models can reproduce the transcriptional status of immune cells in human atherosclerosis. They perform an exhaustive analysis of the transcriptional signature of myeloid populations in murine atherosclerotic lesions by integrating 12 scRNAseq studies encompassing both male and female LDLR^{-/-} and Apoe^{-/-} and PCSK9 AAV models of murine atherosclerosis to generate a total of 18,287 myeloid cells across the studies, providing a strong and compelling base for their findings. The extensive number of cells included provide high confidence in the murine data and establishes a valuable murine atherosclerosis immune cell atlas for the field. Their murine integrated analysis showed several populations of dendritic cells, monocytes and most importantly macrophages. The dendritic cells include 3 distinct populations; cDC1, cDC2 and a Mature cDCs with immune-regulatory signature populations, while macrophages are separated into 11 different clusters which can be grouped under the broad designation of resident/resident-like, inflammatory, foamy, interferon-inducible (IFNIC) and proliferating macrophages (Figure 1).

Finally, the authors try to address an essential question about the translational relevance of the murine data to human disease. They re-analysed some of the available human data to identify the same populations through a mouse and human integrated study. Unlike the extensive murine datasets available to the authors, the human myeloid dataset comprised a total of 2,890 cells collected from 11 patients from 2 studies, one comprising 4 specimens of human coronary artery disease[3] and a second study of 7 carotid endarterectomy

specimens[4]. This analysis demonstrates a superposition of human to mouse myeloid cell phenotypes and show that human myeloid cells from atherosclerotic plaque do fit the broad categories identified in murine models, including a resident-like, inflammatory, foamy, IFNIC and proliferating macrophages (Figure 1).

TLF (Timd4-Lyve1-Folr2) or resident macrophages are usually placed in the adventitia and are CSF1-dependent. Their deep topographic and functional interaction with smooth muscle cells is vital for the homeostasis and optimal arterial diameter and the ECM content of the arterial wall[5]. Their homeostatic role of cholesterol efflux and immune-regulation makes them a force for the good during atherogenesis[6]. We and others identify the 2 main TLF subsets that can be distinguished by the presence or absence of CD209, albeit the functional role of the two subsets is unknown[6,7]. There is further heterogeneity in the murine TLF macrophage population which encompasses at least 3 distinctive subsets in our study[6], including HMOX1+ macrophages[8]. In humans LYVE1+ macs are also identified, suggesting they may have a role also in human atherosclerosis.

Inflammatory macrophages include an overt inflammatory subset with inflammasome genes and a subset with chemokine signature. A murine equivalent inflammasome signature population is identified in the human integrated analysis. Cycling macrophages formed 2 populations in the murine analysis separated through their proliferating cycle.

CD11c expression is a feature of arterial intimal myeloid cells, as well as adipose tissue macrophages. Intimal CD11c+ myeloid cells have been always recognised as important in atherogenesis in the work of Cybulsky and collegues[9]. Their deletion via CD11c-DTR reduces the atherogenic burden[9,10]. CD11c+ macrophages can be distinguished from intimal dendritic cells using multi-analyte approaches in human and mouse[7]. Williams and colleagues demonstrates CD11c+ aortic intima resident macrophages (Mac[Air]) to be CSF-1 dependent and of embryonic origin[10]. They are derived from the ductus arteriosus and have a way of preventing thrombosis by filling endothelial gaps in the arteries[11]. Once atherogenesis starts, embryonic Mac[Air] accumulate lipids and expand through proliferation however, as disease progresses, they are substituted by their bone marrow derived counterpart[10]. The key finding of the paper by Zermecke et al is the clear resolution of the heterogeneity of *Itgax* (gene encoding for CD11c) macrophage populations, which is of great interest. They show through a higher resolution that this population is made up of 3 subpopulations comprising Mac[Air], and 2 population with the highest expression of *Trem2*, one expressing Slamf9 and a second population expressing *Gpnmb. Gpnmb/TREM2* population displays the highest foamy-associated gene signature and is the only expanding

CD11c+ population during atherosclerosis progression. In humans, TREM2^{hi} macrophages express *GPNMB*, suggesting a more evolved transformation towards foamy macrophages, while the embryonically-driven Mac^{Air} was not identifiable.

In summary, this manuscript provide a reliable description of murine myeloid composition in atherosclerosis. It also provides a preliminary framework for human cells in the disease, and highlights the need for more in depth analysis of human and murine data, while detailing heterogeneity of the TREM2 population which is of great interest to the field. The resolution of human atherosclerotic immune cells in the study is definitely inferior than the mouse. This is expected because single cell biology is a numbers game and a higher number of events will be needed to fully resolve the heterogeneity of the myeloid populations in humans and draw a full comparison. The question remains on how similar these populations are when higher resolution is provided, a question that can only be answered when additional human studies with more cells/patients become available. Single cell biology gives us a great opportunity to capture the transcriptional states of immune cells within lesions and to identify better models for human disease, both *ex vivo* and *in vivo* by deciphering myeloid heterogeneity and its role in disease.

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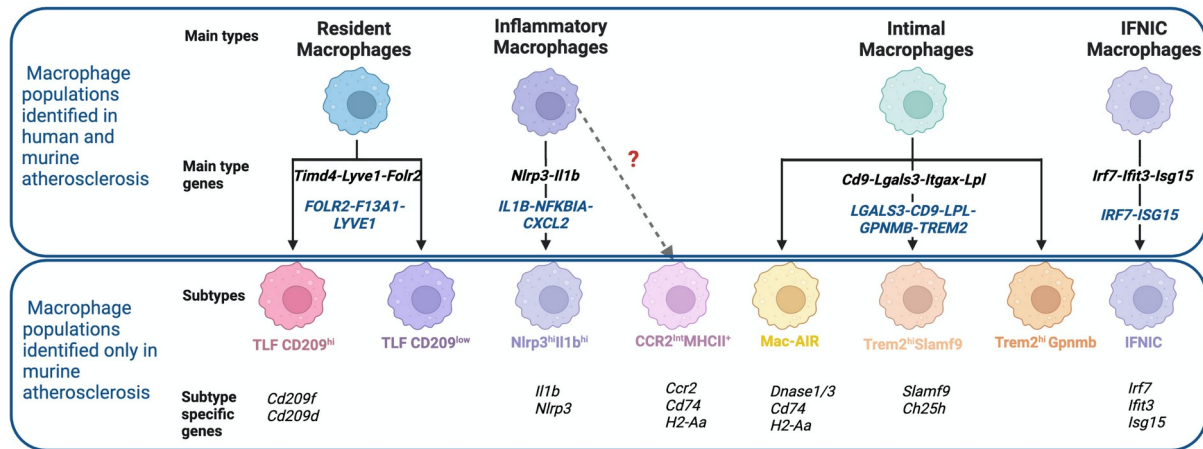
Figure 1. Classification of murine macrophage populations in atherosclerosis and their human equivalent using scRNA seq. analysis. Four (Resident, Inflammatory, Intimal and IFNIC) main macrophage populations are common in murine and human models of atherosclerosis. They are identified with distinct gene markers (black for murine, blue for human). Each main murine subset is further divided into 1 or more subtypes with selective gene expression. Human subtype equivalents are yet to be identified. (Figure was created with Biorender.com).