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EDITORIAL

Macrophage Heterogeneity and Efferocytosis: Beyond the M1/M2 Dichotomy

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Macrophage heterogeneity plays a pivotal role in the complex landscape of atherosclerotic cardiovascular disease, influencing plaque development. The role of macrophages in atherosclerotic plaque formation is multifaceted, involving inflammation, efferocytosis, and even mineral resorption by macrophage-derived osteoclasts.[1] Macrophages, particularly the classically activated M1 subtype, contribute to inflammation by releasing proinflammatory cytokines and actively participating in the formation of atherosclerotic lesions. M1 macrophages populate growing plaques, promoting a proinflammatory microenvironment and facilitating the accumulation of cholesterol.[2,3] Efferocytosis, on the other hand, is a crucial process mediated by macrophages that involves the clearance of apoptotic cells within the plaques. As atherosclerosis progresses, cell death occurs within the lesions, leading to the presence of apoptotic cells. Macrophages, especially the alternatively activated M2 subtype, play a key role in recognizing and engulfing these apoptotic cells through efferocytosis. Failure in efficient efferocytosis results in secondary necrosis, further fueling inflammation, necrotic core formation, and plaque instability.[4,5] The balance between macrophage-mediated inflammation and efferocytosis is crucial for the dynamic nature of atherosclerotic plaque buildup. Dysregulation in this equilibrium can lead to the formation of unstable plaques, increasing the risk of rupture and subsequent cardiovascular events, particularly in the presence of microcalcifications. Understanding and targeting these macrophage-mediated processes offer potential avenues for therapeutic interventions in atherosclerosis.

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Two decades ago, Mills and colleagues introduced the M1/M2 terminology.[6] The concept stemmed from observing variations in arginine metabolism between macrophages from C57BL/6 and Balb/c mice, a phenomenon they associated with distinctions in Th1 and Th2 cell responses within the same strains. In recent years, we have learned that the concept of the M1/M2 dichotomy oversimplifies the pattern of more complex macrophage heterogeneity because the combinatorial spectrum of these cellular subpopulations is wide.[7] With novel technical advancement allowed for multiple parametric assessments, including FACS, CyTOF, single-cell RNA sequencing, and network medicine, a multidimensional model has emerged.[8] Recent studies delineating the single-cell immune landscape of human atherosclerotic plaques have identified several distinct clusters of plaque macrophages and revealed a greater functional heterogeneity compared with the classical concept of M1/M2 dichotomy.[9] Using primary human macrophages activated by interferon gamma (IFNγ) or M(IFNγ), Decano et al[10] employed an integrated approach involving single-cell RNA sequencing, time-course cell-cluster proteomics, metabolite consumption analysis, immunoassays, and functional tests to identify at least 2 major functional macrophage clusters within M(IFNγ). A proinflammatory subset of macrophages showed increased expression of inflammatory chemokines and higher amino acid consumption, while phagocytic macrophages exhibited enhanced phagocytic, efferocytotic, and chemotactic capabilities with elevated Krebs cycle activity and reduced glycolysis.[10] The emerging field of functional macrophage heterogeneity continues to expand our understanding of immune responses in health and disease and translation into the clinic.

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In the atherosclerotic plaque, for macrophages to perform efficient efferocytosis, it requires the interplay of 3 components: (1) receptors on macrophages (TAM receptors, including Tyro3 [TYRO3 Protein Tyrosine Kinase], Axl [AXL receptor tyrosine kinase], and MerTK [MER Proto-Oncogene, Tyrosine Kinase]), (2) bridging molecules (eg, Gas6), and (3) eat-me signals on apoptotic cells (Figure). The TAM receptors are a family of receptor tyrosine kinases with shared ligands—Gas6 and Protein S. The TAM receptors bind the eat-me signal phosphatidylserine on apoptotic cell membranes using Gas6 as a bridging ligand. It is well known that there is differential polarization and the expression of efferocytosis receptor MerTK on M1 and M2 macrophages isolated from coronary artery disease patients.[11] Gas6 particularly is a fascinating target

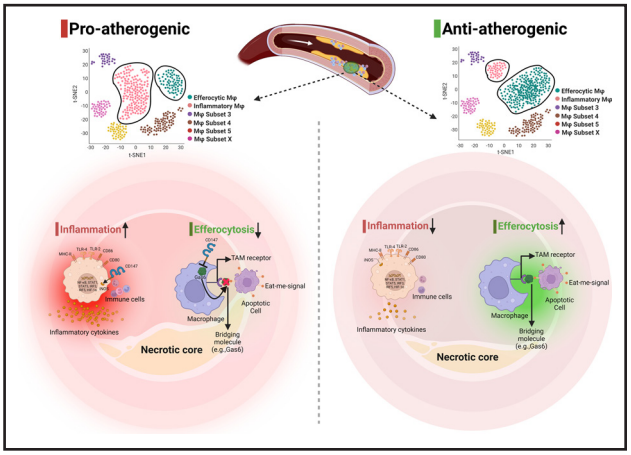

Figure. Macrophage heterogeneity and necrotic core formation.
The formation of a necrotic core within an atherosclerotic plaque is influenced by a delicate balance between inflammation and efferocytosis. Heterogeneous macrophage subpopulations, each characterized by distinct functional phenotypes, are revealed within atherosclerotic plaques through single-cell sequencing. The existence of one or more subset populations within a specifically activated macrophage subset, coupled with an imbalanced representation of another subset, can significantly shape the trajectory of disease progression. Under proatherogenic conditions (left), a larger necrotic core forms due to 2 primary factors. First, there is an expansion of the subset of inflammatory macrophages, releasing an excess of inflammatory factors, orchestrating the recruitment of immune cells, and perpetuating an inflammatory milieu. Secondly, there is a reduction in the subset of efferocytic macrophages, leading to inadequate clearance of apoptotic cells. Lv and colleagues demonstrated that CD147 promotes proatherogenic conditions via regulation of inflammation and efferocytosis (**left**). Conversely, in an antiatherogenic environment (**right**), the opposite unfolds. Understanding and controlling of the delicate equilibrium between inflammation and efferocytosis represent focal points of research aimed at devising therapeutic strategies to stabilize atherosclerotic plaques and mitigate the risk of complications linked to plaque rupture. Strategies that bolster efferocytosis or temper excessive inflammation emerge as promising avenues for intervening in atherosclerosis. CD indicates cluster of differentiation; Gas6, growth arrest specific-6; Mφ, macrophage; MHC, major histocompatibility complex; iNOS, inducible nitric oxide synthase; TAM, Tyro3, Axl, and MerTK; and TLR, toll like receptors.

as clinical evidence associated its circulating levels with reduced human carotid atherosclerotic plaque burden in high-risk cardiac patients.[12] In macrophages, STAT6 induces expression of Gas6, which clear apoptotic bodies and resolves inflammation.[13] Proatherogenic conditions may have less Gas6 expression resulting in impaired efferocytosis and increased necrotic core size.

In the current issue of *Circulation Research*, Lv et al[14] present a novel function of CD147 in destabilizing atherosclerotic lesions by promoting the M1 macrophage phenotype and hindering apoptotic cell clearance. The investigation specifically targeted myeloid-derived CD147 in atherosclerosis and its translational implications. Executed primarily in *Apoe*−/− mice, the study revealed that myeloid-specific CD147 deletion mitigates inflammation and atherosclerosis. Corresponding in vivo data indicated that macrophages from deficient mice undergo a phenotypic shift from proinflammatory to anti-inflammatory in response to LPS (lipopolysaccharide)/IFN-γ, displaying reduced iNOS-derived nitric oxide and reactive nitrogen species. The TRAF6-IKK-IRF5 (TNF Receptor Associated Factor 6-Inhibitor of nuclear factor-κB Kinase-Interferon Regulatory Factor 5) signaling pathway is mechanistically crucial for the impact of CD147 on proinflammatory responses. Myeloid-specific CD147 deficiency reduced the necrotic core size, decreasing susceptibility to iNOS-mediated late apoptosis and enhancing efferocytotic capacity through increased Gas6 secretion in proinflammatory macrophages. These outcomes hold true in a mouse model with myeloid-restricted CD147 overexpression. The authors employed an elegant atherosclerosis model in *Apoe*−/− mice with humanized CD147 transgenic expression and demonstrated that the administration of the anti-human CD147 antibody effectively suppressed atherogenesis by targeting inflammation and efferocytosis. The research establishes the pivotal role of myeloid CD147 in plaque growth by promoting inflammation and inhibiting efferocytosis during proinflammatory conditions (Figure, left). Consequently, anti-human CD147 antibodies present a potential therapeutic avenue alongside existing lipid-lowering strategies for atherosclerotic diseases.

Lv and colleagues showed changes in collagen or a reduction in the necrotic core. Future investigations, however, are necessary to assert whether CD147 can indeed destabilize plaques using specific technologies to assess tissue integrity including finite element analysis. Considering our current knowledge that macrophage heterogeneity is more complex than M1/M2, an in vitro concept represented by M1 phenotype may not fully encapsulate the complexities of plaque biology. This study clearly reported that CD147-targeted therapy suppressed the M1 phenotype. It would be interesting to address whether suppressing CD147 could expand anti-inflammatory macrophage subpopulations, which is critical information for the development of safe therapeutics. Macrophages are diverse, and the intricate aspects

of real plaque biology reflecting their spectrum currently can only be answered using single-cell RNA sequencing.

Lv and colleagues provided convincing evidence to outline mechanisms by which CD147 regulates inflammation and efferocytosis. Further examinations may identify what lies in upstream regulation and downstream effects of CD147. Is there a link between the CD147-mediated inflammation via TRAF6-IKK-IRF5 signaling and efferocytosis via STAT6 mechanisms? Is Gas6 the important link between macrophage inflammation, efferocytosis, and plaque progression? The authors performed clever experiments to show that anti-human CD147 antibody effectively suppressed atherosclerosis by targeting inflammation and efferocytosis in CD147 humanized mice. CD147, however, is a member of the immunoglobulin superfamily and plays fundamental roles in intercellular recognition involved in various immunologic processes. Concerns regarding potential adverse effects due to immunosuppression and increased susceptibility to infections need to be ascertained.

In essence, Lv and colleagues study marks a significant advancement in the understanding of the functionality of CD147 in atherosclerosis, specifically in the regulation of inflammation and efferocytosis. The clinical implications of this study are yet to be established. These intriguing prospects emerge from Lv and colleagues' research and warrant focused investigation in subsequent studies within this domain.

ARTICLE INFORMATION

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Disclosures

None.

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