Atherosclerosis: Open Access

Macrophages in Atherosclerosis: Sources, Functions, Phenotypes, and Therapeutic Strategies

Mahira Munawar*

Department of Cardiovascular Medicine, Aga Khan University, Karachi, Pakistan

Introduction

Atherosclerosis, an enduring inflammatory condition affecting arteries [1], is characterized by the thickening of the arterial intima, proliferation of smooth muscle cells (SMCs), accumulation of lipids, and the formation of plaques. It primarily occurs in regions with disturbed non-laminar flow and damage to the endothelial cells [2]. A multitude of immune cells is believed to play a significant role in the initiation and progression of atherosclerosis, including monocytes, macrophages, dendritic cells, T cells, and B cells [3]. Among these immune cells, macrophages are crucial contributors to atherosclerosis and are the predominant cell type found within atherosclerotic plaques [4]. Macrophages exhibit distinct functional phenotypes; pro-inflammatory M1 macrophages are typically found near the lipid core, while anti-inflammatory M2 macrophages are more abundant in neo-angiogenic regions [5]. Research suggests that the phenotype of macrophages can influence the remodeling of atherosclerotic lesions [6]. Hence, targeting macrophages, regulating their phenotype, and utilizing them or their components as drug carriers hold great promise in the treatment of atherosclerosis. In this review, we delve into the functions and phenotypes of macrophages in atherosclerotic lesions, and we explore potential therapeutic approaches involving engineered macrophages and drug delivery systems.

Macrophages in atherosclerosis: Macrophages play a central role in the progression of atherosclerotic inflammation and are the predominant immune cell type found within atherosclerotic plaques. These macrophages originate from three main sources: (i) circulating monocytes, (ii) vascular smooth muscle cells (SMCs) that undergo transdifferentiation, and (iii) artery-resident macrophages. The primary source of macrophages in atherosclerosis is circulating monocytes, which are recruited by activated endothelial cells and differentiate into macrophages under the influence of macrophage colony-stimulating factor (M-CSF) [7,8]. The content of macrophages within the plaques is directly correlated with the number of monocytes, and increased monocytosis has been shown to accelerate the progression of atherosclerosis [9,10]. Monocytes are primarily produced through medullary hematopoiesis, where hyperlipidemia stimulates the proliferation of hematopoietic stem and progenitor cells, leading to elevated monocyte levels in circulation. Additionally, extramedullary hematopoietic function, particularly in the spleen, has also been found to contribute to atherosclerosis by increasing the number of monocytes derived from the bone marrow and maturing in the spleen. Vascular smooth muscle cells (SMCs) are another significant cell type found throughout the stages of atherosclerotic plaque development. Studies have shown that SMCs, when exposed to cholesterol, can transdifferentiate into macrophage-like cells, with downregulation of SMC markers and upregulation of macrophage-like markers. These foam cells derived from SMCs have lower expression of ATP-binding cassette transporter A1 (ABCA1) and weaker cholesterol transport capacity compared to myeloid lineage cells. It has been demonstrated that over 50% of foam cells in human atherosclerotic plaques originate from SMCs. Recent research utilizing SMC-lineage tracing in ApoE/ mice suggests that the contribution of SMCs to total foam cells is comparable to leukocyte-derived foam cells. However, further investigations are needed to fully understand the mechanisms and contributions of SMC-derived macrophage-like cells to atherosclerosis progression. Apart from circulating monocytes and SMCs, artery-resident macrophages represent another source of atherosclerotic macrophages. These macrophages originate embryonically from precursors expressing C-X3-C motif chemokine receptor 1 (CX3CR1) derived from bone marrow monocytes. These precursors migrate to the blood vessel wall and settle shortly after birth. The specific role of these resident macrophages in atherosclerosis is not yet fully clear, but it is speculated that some of them may take up lipids and transform into foam cells. Further research is required to elucidate their precise contribution to the progression of atherosclerosis. Macrophages within atherosclerotic plaques exhibit diverse functions based on their origin and phenotype. The crucial role of oxidized low-density lipoprotein (oxLDL) in atherosclerosis involves its interaction with endothelial cells, macrophages, and smooth muscle cells (SMCs). In the early stages of atherosclerosis, the primary function of macrophages is to internalize and break down sub-endothelially retained lipoproteins. They accomplish this by engulfing oxidized low-density lipoproteins through scavenger receptors like CD36, SR-A1 (MSR1), scavenger receptor B1 (SR-B1), LDL receptor-related protein 1 (LRP-1), and lectin-like oxLDL receptor-1 (LOX-1). The internalized oxLDL is then hydrolyzed into cholesterol and free fatty acids by lysosomal acid lipase. Some of the free cholesterol is converted into cholesteryl esters by cholesterol acyltransferase-1 (ACAT-1) and stored as lipid droplets in the endoplasmic reticulum of macrophages. The remaining free cholesterol can be expelled from the macrophages through cholesterol ATP-binding cassette (ABC) transporters, including ABCA1, ABCG1, and SR-B1. However, under pathological conditions such as high blood pressure, smoking, diabetes, and high levels of oxLDL, macrophage metabolism becomes impaired, leading to the accumulation of cholesteryl esters within the macrophages and eventually resulting in the formation of foam cells. Moreover, they secrete chemokines and cytokines that promote vascular inflammation, such as monocyte chemoattractant protein-1 (MCP-1), interleukins (IL-1, IL-6, IL-12, IL-15, IL-18, IL-23), and tumor necrosis factor-alpha (TNF-a). These surface molecules function as immune recognition receptors, activating the acquired immune response pathway and amplifying the local inflammatory response. Macrophages in atherosclerosis can be activated by oxLDL through toll-like receptors and the nuclear

translocation of NF-jB. The interaction between oxLDL and CD14-TLR4-MD2 triggers cytoskeletal rearrangement and the production of TNF-a, IL-6, and IL-10. Additionally, cholesterol crystals within foam cells can activate the NACHT, LRR, and PYD domain-containing protein 3 (NLRP3) inflammasome, leading to the release of IL-1b. Hence, targeting the macrophage response to lipids and pro-inflammatory cytokines holds potential as a therapeutic approach for atherosclerosis treatment. Macrophage apoptosis is a crucial process contributing to the formation of the necrotic core, a key characteristic of vulnerable plaque lesions in atherosclerosis. The factors leading to macrophage death in atherosclerosis include oxidative stress, high levels of cytokines, oxidized low-density lipoprotein, apoptosis induced by Fas ligand, and endoplasmic reticulum stress. Endoplasmic reticulum stress is strongly associated with macrophage death and the formation of atherosclerotic necrotic cores, as it activates the unfolded protein response (UPR) and subsequently triggers the activation. Macrophages also play a significant role in the formation of vulnerable plaques, which can eventually lead to plaque rupture. Matrix metalloproteinases (MMPs) secreted by macrophages are involved in the thinning of the fibrous cap and the development of vulnerable plaques. Macrophages and activated MMPs, such as MMP-2 and MMP-9, are found in the shoulder region of unstable atherosclerotic plaques. MMP-9, in particular, is implicated in various stages of atherosclerosis and facilitates macrophage infiltration into the lesion, thereby promoting early-stage disease progression. Other MMPs, such as MMP-1, MMP-3, MMP-8, MMP-13, and MMP-14, also contribute to plaque instability, each exerting somewhat different effects. The degradation of the extracellular matrix (ECM) by MMPs is considered a likely factor contributing to plaque instability and rupture. Additionally, other proteases, such as cathepsin K, play a role in the development of atherosclerosis. Cathepsin K induces atherosclerosis by regulating ECM redistribution, and its expression is influenced by the disturbed flow-mediated integrin-cytoskeleton-NF-jB signaling axis. In the context of the immune response, macrophages have essential roles in both innate and adaptive immunity. They act as antigen-presenting cells, presenting major histocompatibility complex class I (MHC-I) molecules to naive CD8+ T cells and MHC-II molecules to naive CD4+T cells. CD8+ killer T cells induce apoptosis and necrosis of target cells through cytotoxins or cytokines, thereby exacerbating the inflammatory response in atherosclerotic plaques and driving lesion progression and instability. CD4+ T cell subsets can differentially affect the progression of atherosclerosis through immune activation or immunosuppression or by assisting B cells in antibody production.

Conclusion

Macrophages play a crucial role in the development of atherosclerosis, and their functions and phenotype are influenced by their microenvironment. However, the formation of foam cells and chronic inflammatory responses in lesion areas contribute to disease progression. Therefore, understanding and controlling macrophage phenotype and functions in atherosclerosis are critical for therapeutic advancements. To gain deeper insights into macrophage phenotypes and their interactions with other immune and vascular cells, further analysis at the single-cell level is necessary. Recent single-cell sequencing studies of immune cells in human aortic plaques have revealed that macrophages and T cells are the predominant immune cell types, with macrophages accounting for about 16% of the plaque composition. Subsequent analyses of macrophages have revealed significant functional heterogeneity, including clusters with activated and proinflammatory phenotypes, as well as a cluster exhibiting a foam cell transcriptional signature. Combining single-cell sequencing with spatial transcriptome analysis allows for cellular profiling with higher temporal and spatial resolutions, enabling the study of dynamic changes in macrophage phenotype and cell-cell interactions within the lesion area. These findings provide valuable insights into new mechanisms and key factors that regulate macrophage phenotype and offer potential therapeutic targets. In addition to discovering novel therapeutics, the optimization of delivery platforms is essential. Intravenous injection of nanotherapeutics is an effective systemic approach for their infiltration into atherosclerotic plaques. However, the formation of a nanoparticle protein corona, influenced by proteins in the blood, can impact their pharmacokinetics, cellular uptake, and bio distribution. Current preclinical studies focus on prolonging carrier circulation time by surface modification using materials like PEG or biomimetic components such as macrophage membrane, erythrocyte membrane, platelet membrane, and exosomes. Strategies that enhance nanoparticle accumulation in diseased macrophages include passive extravasation of leaky vasculature and active targeting combined with endocytosis. Novel approaches, such as synthetic biology to design liposome-based delivery systems or scaling up cell membrane component production for nanoparticle coating, can offer potential solutions.

Moreover, the size, shape, charge, chemical composition, surface functional groups, and targeting ligands of nanoparticles significantly influence their biodistribution within plaques. Studies have shown that smaller nanoparticles with specific shapes, like rods and cylindrical nanocarriers, have greater vascular tissue penetration compared to larger spherical nanoparticles. However, more research is needed to explore the effects of these nanoparticle characteristics on their distribution in plaques systematically. Finally, genetically engineered immune cell therapies, which have shown promise in cancer treatment, offer potential for innovative immunotherapy in atherosclerosis. There is an opportunity to engineer macrophages and other immune cells to possess desirable phenotypes and functions, paving the way for innovative immunotherapy approaches to tackle atherosclerosis.

Acknowledgement

Conflict of Interest

Author declares no conflict of interest.

References

1. Koskinen P, Manttari M, Manninen V, Huttunenctk, Heinnonen OP, et al. (1992) Coronary heart disease incidence in NIDDM patients in the Helsinki Heart Study. Diabetes Care 15:820-825.

2. Van Ark J, Hammes HP, Van Dijk MC, Lexis CP, Van Der Horst IC, et al. (2013) Circulating alpha-klotho levels are not disturbed in patients with type 2 diabetes with and without macrovascular disease in the absence of nephropathy. Cardiovasc Diabetol 12:116.

3. Oberman A, Kouchoukos NT, Holt JH, Russell RO (1977) Long-term results of the medical treatment of coronary artery disease. Angiology 28:160-168.

4. Ishii H, Jirousek MR, Koya D, Tagaki C, Xia P, et al. (1996) Amelioration of vascular dysfunction in diabetic rats by an oral PKC inhibitor. Science 272:728-731.

5. Kannel WB, Abbott RD (1984) Incidence and prognosis of unrecognized myocardial infarction. An update on the Framingham study. N Engl J Med 311:1144-1147.

6. American Diabetes Association (2004) Nephropathy in diabetes. Diabetes Care 27:79-83.

7. Bansal S, Wackers FJT, Inzucchi SE, Chyun DA, Davey JA, et al. (2011) Five-year outcomes in high-risk participants in the Detection of Ischemia in Asymptomatic Diabetics (DIAD) study: a post hoc analysis. Diabetes Care 34: 204-209.

8. Nesto RW, Phillips RT, Kett KG, Hill T, Perper E, et al. (1988) Angina and exertional myocardial ischemia in diabetic and nondiabetic patients: assessment by exercise thallium scintigraphy. Ann Intern Med 108:170-175.

9. Baber U, Bander J, Karajgikar R, Yadav K, Hadi A, et al. (2013) Combined and independent impact of diabetes mellitus and chronic kidney disease on residual platelet reactivity. Thromb Haemost 110:118-123.

10. Seshasai SR, Kaptoge S, Thompson A, Di Angelantonio E, Gao P, et al. (2011) Diabetes mellitus, fasting glucose, and risk of cause-specific death. N Engl J Med 364:829-841.