Macrophages and Atherosclerosis

Subjects: Immunology

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Macrophages are the key inflammatory cell type involved in all stages of atherosclerosis development and progression, as demonstrated by numerous studies. Correspondingly, macrophages are currently regarded as a promising therapeutic target for the development of new treatment approaches. The macrophage population is heterogeneous and dynamic, as these cells can switch between a number of distinct functional states with pro- and anti-atherogenic activity in response to various stimuli. An atherosclerotic plaque microenvironment defined by cytokine levels, cell-to-cell interactions, lipid accumulation, hypoxia, neoangiogenesis, and intraplaque haemorrhage may guide local macrophage polarization processes within the lesion.

atherosclerosis macrophage intraplaque

1. Introduction

Atherosclerosis is currently recognized as a chronic inflammatory condition, with inflammatory cell types tightly involved in its initiation and progression. The hallmark of chronic atheroinflammation is progressive accumulation of lipids and inflammatory cells in the affected sites of the arterial wall [1]. At the early stages of atherogenesis, plasma low-density lipoprotein (LDL) enters the subendothelial space of the arterial wall, forming lipid deposits. This occurs preferentially at sites of laminar blood flow perturbations, such as bends and bifurcations of the vessel, and/or in the areas of local endothelial dysfunction [1]. Modified LDL, such as oxidized LDL (oxLDL), is especially atherogenic. It is currently assumed that oxidation of accumulated LDL may take place in the arterial wall, with formation of oxidation-specific epitopes which are recognized by the innate immune system, leading to immune activation. These molecules are known as “danger-associated molecular patterns” (DAMPs) [2]. These early events trigger the immune response that involves many cellular subtypes of both innate and adaptive immunity [2]. However, among these various cell types, macrophages and T lymphocytes are the major subsets of inflammatory cells in atherosclerotic lesions [3]. Macrophages were found to be present at all stages of plaque formation, and are currently recognized as a cell type orchestrating the complex process of atheroinflammation.

Macrophages internalize LDL and very low-density lipoprotein (VLDL), as well as oxidized and other modified lipoproteins by means of macropinocytosis, phagocytosis, and scavenger receptor-mediated uptake. This leads to metabolic and functional reprogramming of the cell [3], with decreased phagocytic activity, increased production of pro-inflammatory cytokines, and transformation into foam cells [4]. This type of cell received its name because of the foam-like appearance of the cytoplasm filled with accumulated lipid droplets, and is abundantly present in growing atherosclerotic plaques. In particular, foam cells constitute the primary cell population of fatty streaks, the initial stage in atherosclerosis plaque development. Foam cell formation may be a beneficial adaptive process at

the early stages of atherosclerosis, but it appears to be detrimental in advanced lesions due to the massive death of foam cells and release of their pro-inflammatory contents, decreasing plaque stability [5]. Macrophages, as well as dendritic cells, are involved in antigen presentation to T cells, therefore activating the Th1 (T-helper type 1) response and promoting inflammation and atheroma progression. In turn, Th1 cells stimulate pro-inflammatory activation of macrophages by creating a specific cytokine environment [1]. These feedback connections establish a pro-atherogenic signaling cascade responsible for plaque development. Finally, macrophages influence plaque stability by regulating collagen production, the release of matrix degrading enzymes, and induction of smooth muscle cells (SMCs) apoptosis [5]. Even dead macrophages continue to participate in atherosclerosis pathogenesis [6]. At late stages of plaque progression, macrophages contribute to the development of plaque necrotic scores, therefore amplifying the inflammatory response by excessive cell death in the context of defective cell clearance [6].

In an atherosclerotic plaque environment, macrophages are exposed to various signals and stimuli, including cytokines, modified lipids, senescent erythrocytes, and hypoxia, that influence their transcriptional program and functional phenotype. The intensity of these signals changes during plaque progression, and also varies between the plaque regions. As a consequence, intraplaque macrophages undergo polarization in distinct subtypes, often playing opposite roles in atherosclerosis pathology. The concept of macrophage polarization provides a framework for the understanding of the function of macrophages in atherosclerosis development and creates a new paradigm for macrophage targeting for therapeutic purposes.

2. Single-Cell Level of the Diversity of Intraplaque Macrophages

The discovery and rapid progress of single-cell RNA sequencing (scRNAseq) and tools exploiting large combinations of protein labelling of cells, such as cytometry by time of flight (CyTOF), have now allowed classification of macrophages based on transcriptional patterns or protein marker sets and thus provided a more detailed description of intraplaque macrophage phenotype and functional diversity.

Studies of atherosclerosis development in *Ldlr*$^{-/-}$ or *ApoE*$^{-/-}$ mice with CyTOF or scRNAseq approaches confirmed previous histological findings, showing that macrophages are the most prevalent immune cell type within the atherosclerotic plaque [7][8] and the total proportion of macrophages increases with the evolution to more advanced stage [8]. Myeloid cells, including macrophages and monocytes, were underrepresented in digestion-based scRNAseq and flow cytometry studies, with CD11b^{+} cells comprising 20% of plaque leukocytes. At the same time, the genetic deconvolution method gave a more representative picture of plaque composition, showing the frequency of myeloid cells of more than 75% [9]. Summarizing currently available single-cell data from murine atherosclerosis models, three main subtypes of intraplaque macrophage could be identified, including resident-like macrophages, pro-inflammatory and foamy triggering receptor expressed on myeloid cells (TREM)2hi macrophages (**Table 1**). Further subdivision of these three main types is possible [7].

Table 1. Classification of murine intraplaque macrophages according to single-cell analysis.

Type	Resident-Like Macrophages	Inflammatory Macrophages	TREM2hi Macrophages
Markers	Lyve1, Cxcr1, Folr2, Cd206, F13a1, Cbr2, Sepp1, Cxcl4, Gas6, Mafb	Tnf, Nlrp3, Il1b, Egr1, Cepbp, Cxcl1, Ccl2–5, Nfkbia	Trem2, Cd9, Lgals3, Ctsb, Spp1
Functional pathways	Endocytosis, proliferation, anti-inflammatory	Inflammatory response	Cholesterol metabolism, oxidative phosphorylation, lipid accumulation, anti-inflammatory
Localization	Adventitia	Intima, plaque shoulder	Intima, necrotic core
Markers of corresponding population in human atherosclerosis	Cd206, Cd163	Hla-dra, Cd74, Cyba, Lyz2, Aif1, S100A8/A9, Malat1, JunB, Nfkbia	Apoc1, ApoE, Ctsb, Fabp5, Plin2, Lgals3, Trem2, Cd9, Lxr, Stat6

Aif, Apoptosis-Inducing Factor; ApoE, Apoliprotein E; Apoc1, apolipoprotein C1; Cbr2, Carbonyl reductase; Ccl, C-C motif ligand; Cepbp, CCAAT/enhancer-binding protein beta; Ctsb, Cathepsin B; Cxcl4, C-X-C motif ligand 4; Cx3cr1, C-X3-C Motif Chemokine Receptor 1; Cyba, Cytochrome B-245 Alpha Chain; Egr1, Early Growth Response 1; F13a1, factor XIIIa; Fabp5, Fatty Acid Binding Protein 5; Folr2, Folate Receptor Beta; Gas6, Growth Arrest Specific 6; IL1b, Interleukin 1 beta; Lgals3, Galectin-3; Lxr, Liver X receptor; Lyve1, lymphatic vessel endothelial hyaluronan receptor 1; Lyz2, Mafb, MAF BZIP Transcription Factor B; Malat1, Metastasis Associated Lung Adenocarcinoma Transcript 1; Nfkbia, NFKB Inhibitor Alpha; Nlrp3, NLR Family Pyrin Domain Containing 3; Plin2, Perilipin 2; Sepp1, Selenoprotein P; Spp1, Secreted Phosphoprotein 1; S100A8, S100 Calcium Binding Protein A8; Stat6, Signal Transducer And Activator Of Transcription 6; Tnf, Tumor Necrosis Factor, Trem2, Triggering Receptor Expressed On Myeloid Cells 2.

Resident arterial macrophages emerge during embryonic development. They retain expression of the precursor marker CX3C chemokine receptor (CX3CR)1 and specifically express Lymphatic vessel endothelial hyaluronan receptor 1 (Lyve1). Depending on the study, additional markers of this population were identified: mannose receptor MMR (Cd206), transcription factor Mafb [10], Factor XIIIa (F13a1), Growth arrest-specific 6 (Gas6) [8], and others. According to their tissue-resident subtype, these macrophages are identified both in normal and atherosclerotic areas of the aorta [8], and they are predominately present in the adventitia. It is not clear whether migrating monocytes may acquire resident-like phenotype. Increased expression of Ccr2, a marker for recruited macrophages, may point to this possibility. Several studies described that resident-like macrophages can proliferate by showing either protein expression of proliferation marker Ki-67 or enrichment for cell cycle genes [7]. Folr2, Cbr2, Sepp1 and Cd206 expressed by lesion resident-like cells are all associated with M2-like phenotype, suggesting anti-inflammatory characteristics of the population. There was no difference in the proportion of resident-like macrophages between progressive and regressive plaques [7].

Different single-cell studies confirmed the presence of inflammatory macrophages in atherosclerotic lesions [7] and were concordant in the description of this subset. It is enriched with transcripts of classical pro-inflammatory pathways (Il1α, Il1β, Tlr2, Tnf), chemokines (Ccl2–5, Cxcl1, Cxcl2, Cxcl10) and interferon I signaling genes. The relative frequency of the inflammatory macrophage subset positively correlates with plaque progression [7]. A rather unexpected finding was the absence of association of inflammatory patterns with foam cell phenotype (discussed below). In contrast to resident-like population, the inflammatory population is absent in normal vessels, being present only in atherosclerotic lesions, where they represent the largest macrophage subset [8]. They also differ in the localization, with predominant presence in the intima, including plaque shoulder regions [7]. According to the transcriptional and functional profile, this population closely resemble classical M1 phenotype.

TREM2hi macrophages were identified exclusively in the plaques and not in the healthy aorta [8]. TREM2 is a myeloid-specific transmembrane glycoprotein, that works as a lipid sensor, binding apolipoprotein E, glycerophospholipids, sphingomyelins. TREM2 was described as a marker of lipid-associated macrophages differentiated in obesity, driving gene expression program involved in phagocytosis, lipid catabolism and anti-inflammatory phenotype [11]. According to current understanding, TREM2 pathway may represent a conserved macrophage response for detection of extracellular pathogenic lipids across multiple tissues [11]. Pathway analysis of TREM2hi lesion macrophages showed connections with foam cell phenotype and enrichment in transcripts involved in cholesterol metabolism and oxidative phosphorylation [7]. Foam cell characteristics of TREM2hi macrophages were confirmed by independent studies [8][10]. This subset of cells resides in the intima, being involved in the uptake of atherogenic lipoproteins and lipid-rich core formation. Comparison of transcriptomic profiles of intimal foamy and non-foamy macrophages sorted from pooled atherosclerotic aortas of *ApoE*$^{-/-}$ mice showed that inflammatory genes such as *I1b, Nfkbia, Tlr2*, and *Tnf* were mostly upregulated in non-foamy population, while foam cells were enriched in resolving/regression-related genes [10]. The notice that foam cell formation is not a pro-inflammatory process is a recent ongoing paradigm shift in the atherosclerosis field, as several studies have previously shown clear pro-inflammatory characteristics of foam cell formation, which was, for a long time, regarded as a potential target for atherosclerosis treatment [12]. Although foamy macrophages are anti-inflammatory with M2-like features, their accumulation positively correlates with the severity of atherosclerosis [10].

Macrophage subsets defined by Fernandez et al. [13] in human carotid endarterectomy specimens are close to murine classification discussed above. In contrast to murine models, in human plaques, macrophages were not the major population among CD45^{+} cells, comprising less than 20% [14][13]. The study described the same three populations of intraplaque macrophages [13]. The CD206^{hi}CD163^{hi} macrophage subset may be similar to resident-like macrophage subset based on CD206 expression and absence of foam- cell patterns. The pro-inflammatory macrophages in human plaques expressed increased levels of activation markers, such as major histocompatibility complex class II DR alpha (HLA-DRA), CD74, cytochrome B-245 alpha chain (CYBA), lysozyme C-2 precursor (LYZ2), allograft inflammatory factor (AIF)1, S100A8/A9, metastasis associated lung adenocarcinoma transcript (MALAT)1, JUNB, NFKBIA. The foamy anti-inflammatory macrophage subset was enriched for the expression of LGALS3 (galectin 3 gene) and foam cell-related transcripts such as apolipoprotein C (APOC)1, APOE, cathepsin B (CTSB), FABP5 and perilipin 2 (PLIN2). TREM2 and CD9 expression in foamy subset was observed in another study, closely corresponding to the data from murine studies. Characteristics of foamy-like population also included

the upregulation of metabolic pathways and liver X receptor/retinoid X receptor (LXR/RXR) activation, as well as STAT6-driven anti-inflammatory pathways [14].

Macrophage transcriptional profiles may differ between patients with asymptomatic lesions (ASYM) and those having cerebrovascular complications (SYM) [13]. ASYM macrophages were more activated, pro-inflammatory with high IL-1β signaling (IL-1β, IL-1RAP, NLRP3 expression) and displayed enhanced foam cell functions compared to SYM macrophages. In SYM plaques, macrophages expressed several genes associated with plaque instability: granzymes, CCL5, pro-angiogenic factors IL-8 and CXCR2, genes involved in Hedgehog and Wnt signaling. T cell-macrophage and macrophage- macrophage cell-to-cell communications were described that may be associated with ASYM and SYM plaques [13].

Single-cell RNA-Seq was combined with genetic fate mapping of myeloid cells derived from $CX3CR1^{+}$ precursor monocytes to analyze the differences in transcriptional programs between progressing and regressing plaques in mice. Bone marrow chimeras of *ldlr*$^{-/-}$ mice reconstituted with bone marrow from Cx3cr1CreERT2-IRES-YFP/+Rosa26fl-tdTomato/+ mice were used that allow to map all CX3CR1+ macrophages with tdTomato expression upon tamoxifen exposure. No significant difference in the overall number TdTomato+ cells from aortas of mice undergoing progression and regression was found, but regressing lesions were characterized by increased number of macrophages with M2 markers (PD-L2, CD301) [15]. The cluster that resembled resident-like macrophages (Folr2hi macrophages) was the highest in both progressing and regressing lesions. There was a considerable heterogeneity of macrophage activation states under progression conditions possibly reflecting more complex polarizing environment. An interesting finding was the identification of a specific M2 subpopulation ($Retnla^{hi}Ear2^{hi}$ macrophages) in progressing plaques that was actually absent from regressing plaques, that challenged the idea of plaque progression as solely M1-driven process. A specific cluster of M2 polarized cells associated with regressive plaques was also identified, that expressed stabilin-1 (Stab1) and selenoprotein-1 (Sepp1), proteins with efferocytosis-enhancing and anti-inflammatory activities. A cluster of proliferating $CX3CR1^{+}$ cells with stem cell–like signature expressing markers of both monocytes and macrophages was described. This fact broadens our view of the origin of proliferating macrophages within the plaque [15].

3. Conclusions

The current paradigm suggests that M2 macrophages, owing to their specific localization within human atherosclerotic plaques and their intrinsic anti-inflammatory properties, are primarily associated with plaque stability. By contrast, M1 macrophages are predominant in unstable regions, and their role in atheroinflammation is deleterious. However, the real situation may be more complex, with the identification of several pro- and anti-inflammatory functional states with distinct and, in most cases, not fully identified roles in atherogenesis. A direct causative link between macrophage phenotype and progression of atherosclerotic lesions in humans has not been established, with available information limited to correlation studies. There is a high need for the development of new animal models because the clear proof of the concept of macrophages guiding the evolution and regression of plaques may open up a new area in drug development for the treatment and prevention of atherosclerosis. An improved definition of specific macrophage phenotypes and the role they play in atherogenesis may also be useful

for the development of new therapeutic modalities. Due to the high plasticity of macrophages and overlap of macrophage subtype markers, the most perspective strategy seems to be not the targeting or elimination of distinct subpopulations, but modulation of polarizing signals and differentiation pathways, as well as enhancing specific atheroprotective activities.

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