Characteristics of plaque lipid-associated macrophages and their possible roles in the pathogenesis of atherosclerosis

Kyeongdae Kim*, Sang-eun Park*, Ji-su Park and Jae-Hoon Choi

Purpose of review
Recent findings from single-cell transcriptomic studies prompted us to revisit the role of plaque foamy macrophages in the pathogenesis of atherosclerosis. In this review, we compared the gene expression profile of plaque foamy macrophages with those of other disease-associated macrophages and discussed their functions in the pathogenesis of atherosclerosis.

Recent findings
To understand the phenotypes of macrophages in atherosclerotic aorta, many research groups performed single-cell RNA sequencing analysis and found that there are distinct phenotypic differences among intimal foamy, nonfoamy and adventitial macrophages. Especially, the plaque foamy macrophages express triggering receptor expressed on myeloid cells 2 (TREM2), a key common feature of disease-associated macrophages in Alzheimer’s disease, obesity, cirrhosis and nonalcoholic steatohepatitis. These TREM2⁺ macrophages seem to be protective against chronic inflammation.

Summary
As the gene expression profile of plaque foamy macrophages is highly comparable to that of lipid-associated macrophages from obesity, we named the plaque foamy macrophages as plaque lipid-associated macrophages (PLAMs). PLAMs have a high level of gene expression related to phago/endocytosis, lysosome, lipid metabolism and oxidative phosphorylation. Considering the protective function of lipid-associated macrophages against adipose tissue inflammation, PLAMs may suppress atherosclerotic inflammation by removing modified lipids and cell debris in the plaque.

Keywords
atherosclerosis, macrophage, plaque lipid, TREM2

INTRODUCTION
Atherosclerosis is a chronic inflammatory cardiovascular disease. Over the last three decades, the number of cardiovascular disease cases worldwide has nearly doubled, from 271 million cases to 523 million [1], demanding precise risk assessment tools and novel therapeutic regimens. Macrophages are well known primary myeloid cells that accumulate lipids in the cytosol, leading to a foamy appearance of atherosclerotic plaques [2]. Previously, foamy macrophages were assumed to be the pathogenic cell types that drive atherosclerotic inflammation. However, we demonstrated that foamy macrophages in atherosclerotic aorta highly express triggering receptor expressed on myeloid cells 2 (TREM2) and are less inflammatory but possess more homeostatic phenotypes [3]. A recent meta-analysis also recapitulated that foamy macrophages are not inflammatory, but nonfoamy and interferon-inducible macrophages express pro-inflammatory genes.

In this review, we summarized the macrophage populations in normal and atherosclerotic aortas and compared the aortic foamy macrophages with disease-associated macrophages expressing TREM2 in other inflammatory tissues. In particular, we named the intimal foamy macrophages as plaque...
lipid-associated macrophages (PLAMs); this was based on their similarity of gene expression profiles compared with lipid-associated macrophages (LAMs) recruited in adipose tissue during obesity [4**]. Finally, we discussed the possible role of PLAMs in the pathogenesis of atherosclerosis.

REDEFINING THE CHARACTERISTICS OF INTIMAL MACROPHAGES ASSOCIATED WITH PLAQUE LIPIDS

In the steady state, aortic macrophages mostly reside in the adventitia, and a small number of macrophages accumulate in atherosclerosis-prone areas [5–7]. Intimal macrophages residing in atherosclerotic-prone areas are responsible for initial foamy macrophages but are soon replaced by macrophages derived from blood monocytes [7]. During the initial stage of atherosclerosis, plaque macrophages originate mostly from blood monocytes, while some originate from extramedullary monocytes [8]. The infiltration of monocytes depends on the expression of C-C chemokine receptor type 2 (CCR2), CCR5 and CX3CR1 [9,10] and various adhesion molecules on endothelial cells [11]. The recruited intimal macrophages take up modified lipids, mostly oxidized LDL, and have a foamy appearance. Macrophages in atherosclerotic plaques can be affected by various microenvironmental factors, including modified lipoproteins, inflammatory cytokines, cell debris and cholesterol crystals, leading to various cellular phenotypes. For example, the accumulation of lipids can activate liver X receptor (LXR) target genes and suppress sterol regulatory element-binding protein (SREBP) target genes, leading to fatty acid metabolism reprogramming and inflammatory gene suppression [12,13]. In contrast, increased free cholesterol and cholesterol crystals induce NLRP3-dependent activation of macrophages [14–16]. Thus, it is important to examine the macrophage populations in atherosclerotic plaques, the primary site for atherosclerotic inflammation, to understand the pathogenesis of atherosclerosis and develop novel therapeutic immune targets.

Previously, to characterize the phenotype of plaque foamy macrophages, a direct analysis of lesional macrophages using laser capture microdissection [17] or an analysis of foamy macrophages in sponges surgically inserted into the subcutaneous area of hyperlipidemic mice, which showed enhanced gene expression that is related to growth, proliferation and cholesterol metabolism (e.g. Abca1, Ppary, Rxra, Rxrb and Srebpl), was performed [18]. Single-cell RNA sequencing (scRNA-seq) analysis has broadened our understanding of the phenotypic changes in immune and nonimmune cells during disease progression. As scRNA-seq has been well established, many research groups have performed unbiased transcriptome analysis of immune cells from mouse and human atherosclerotic aortas at the single-cell level (Rahman et al. [19], Winkels et al. [20], Cochain et al. [21], Kim et al. [3], Lin et al. [22] and Fernandez et al. [23]). The meta-analysis of available single-cell transcriptome datasets showed the common macrophage populations in the atherosclerotic aorta and the distinct phenotypic differences among adventitial resident, intimal nonfoamy and foamy macrophages [24]. These single-cell analyses revealed that intimal foamy macrophages are less inflammatory than nonfoamy macrophages and express many homeostatic genes related to cholesterol transport, fatty acid metabolism, phagocytosis, endocytosis and protein metabolism compared with their nonfoamy counterparts [3,25*]. Interestingly, foamy macrophages in atherosclerotic plaques highly express TREM2, a typical membrane receptor commonly expressed in recently reported disease-associated macrophages in various inflammatory tissues, including the brain (damage-associated microglia, DAMs), adipose tissue (lipid-associated macrophages, LAMs) and liver [scar-associated macrophages, SAMs and nonalcoholic steatohepatitis (NASH)-associated macrophages, NAMs] (Table 1) [3,4**,21–23,26–32].

EMERGING ROLE OF TREM2+ MACROPHAGES IN INFLAMMATORY DISEASES

TREM2 is a transmembrane receptor of the immunoglobulin superfamily expressed in the immune cells of various tissues [33**]. TREM2 interacts with various molecules, including lipids, apolipoproteins, lipopolysaccharides, dextran sulfate, DNAs and phospholipids [34–37]. TREM2 activates Syk or PI3K via the formation of heterodimers with DAP12 (TYROBP) or DAP10, respectively [38]. It also regulates cell survival via the mTOR or β-catenin pathways [39–42]. Furthermore, TREM2 enhances phagocytosis, leading to suppression of secondary
Table 1. Characteristics of Trem2<sup>hi</sup> macrophages in various diseased organs

| Tissue        | Aorta                      | Adipose tissue                  | Brain                      | Liver                                     |
|---------------|----------------------------|---------------------------------|----------------------------|-------------------------------------------|
| Disease       | Atherosclerosis            | Obesity                        | Alzheimer’s disease        | Cirrhosis                                |
|               |                            |                                 |                            | Nonalcoholic steatohepatitis (NASH)      |
| Trem2<sup>hi</sup> macrophages | Plaque lipid-associated Macrophages (PLAMs) | Adipose tissue lipid-associated macrophages (LAMs) | Disease-associated microglia (DAMs) | NASH-associated macrophages (NAMs) |
|               |                            |                                 |                            | Scar-associated macrophages (SAMs)       |
| References    | (Mouse)                    |                                 |                            |                                           |
|               | Cochin et al. [21]         | Jaitin et al. [4<sup>**</sup>]  | Keren-Shaul et al. [26]    | Xiong et al. [29]                        |
|               | Lin et al. [22]            |                                 |                            | Seidman et al. [30<sup>**</sup>]        |
| Location      | PLAMs are in atherosclerotic plaques and not present in normal aorta | LAMs surround apoptotic adipocytes (crown-like structures) and are not present in a normal adipose tissue | DAMs surround amyloid beta plaques and are not present in normal brain cortex | The cells are present in hepatic sinusoids [NAMs] or collagen-rich areas [SAMs] |
|               |                            |                                 |                            | They are not present in normal liver     |
| Cell origin   | Blood monocytes            | Blood monocytes                 | Homeostatic microglia      | KC-N: from healthy Kupffer cells KN-RM: from blood monocytes |
| Cell distinction | PLAMs are distinct from adventitia macrophages and intimal nonfoamy macrophages | LAMs are distinct from monocytes and tissue-resident macrophages | DAMs are distinct from monocytes and perivascular macrophages | SAMs and NAMs are distinct from healthy Kupffer cells and blood monocytes |
| Representative enriched genes (mouse) | Abca1, Abcg1, Cd36, Cd63, Cd9, Ctsb/d/1/2/3, Fabp4/5, Hvac1, Itgax, Igals3, Lipa, Merk, Mr1, Npc1, Nr1h3, Spp1, Trem2 | C1qa, C36, C68, C69, Ctb, Ctn, Fabp4, Fabp5, Logls1/3, Lipa, Lpl, Trem2 | Apoe, Axl, Cd36, Cd9, Csf1, Cst7, Cstb/d, Hexb, Itgax, Lpl, Ly2, Spp1, Timp2, Trem2, Tyrobp | KC-N: Aif1, Apo1, Apo, C1qb, Ccl24, Csl1, Clec1b, Clec4f, Clec4n, Csd, Ear2, Fabp7, Fol2, Igf1, Il18bp, Lpl, Mmp12, Pltp, Trem2, Wfc17 |
| KN-RM:        | Apoe, Bcl2/e1b, Cd207, Cd63, Cd74, Cd9, Clec4b1, Cxc3cr1, Cxc114, Fabp5, Gpnmmb, H2-Aa, H2-Eb1, H2-M2, Mmp12, Ms4a7, Pl4, Trem2 | | | |
| Relatively low expressed genes | Il1b, Nlrp3, Mgl2 (vs. intima nonfoamy macrophages) | Ccr2, Il1b, Ly6c2, Lyz2, S100a10 (Loss from monocytes) | Ccr5, Cx3cr1, Pyr12/13, Tnip, Trem119, Selppl (vs. homeostatic microglia) | Cd163, C6 (vs. healthy Kupffer cells) |
|               | Mgl2 (vs. Ly6C<sup>hi</sup> RM) | Runx1/2/3 (vs. blood Ly6C<sup>hi</sup> monocytes) | | |
| Enriched pathways | Cholesterol metabolism | Intracellular metabolism | Endocytosis | Endocytosis |
|               | Lysosome                   | Lysosome                        | Lysosome                  | Lipid catabolism |
|               | Oxidative phosphorylation | Oxidative phosphorylation       | Phagocytosis              | Lysosome                  |
|               | Proteasome                 | Phagosome                       | Regulation of immune       | MHCII presentation |
|               | PPAR signalling            | PPAR signalling                 | response                   | ROS metabolic process |
|               |                             | Sphingolipid metabolism         | Response to wounding       | Tissue remodelling |

KC-N, Kupffer cells in nonalcoholic steatohepatitis; KN-RM, recruited macrophages occupying the Kupffer cell niche; MHCII, major histocompatibility complex II; PPAR, peroxisome proliferator-activated receptor; RM, recruited macrophages; ROS, reactive oxygen species.

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necrosis and pro-inflammatory danger signals, and eventually attenuates the inflammatory response [43–45]. Activation of the TREM2 signalling pathway attenuates toll-like receptor (TLR) and the production of TLR-associated cytokines in macrophages and dendritic cells [43,45,46]. Although the exact mechanisms responsible for TREM2 expression remain to be elucidated, it appears that TREM2 is induced in macrophages in a lipid-rich tissue environment with chronic inflammatory conditions and plays a key role in sensing and processing disease-associated microenvironments. TREM2+ macrophages are present in lipid-rich, hypoxic and chronic inflammatory conditions, including atherosclerosis, obesity, Alzheimer’s disease, cirrhosis and NASH. They contain lipid droplets or amyloid beta originating from the surrounding injured tissues, suggesting that they are involved in the clearance of injured cells and debris, leading to the resolution of inflammation. As expected, the decrease in TREM2 expression impairs the phagocytosis of apoptotic cells, cellular debris and lipoproteins in microglia, suggesting the protective function of DAMs against Alzheimer’s disease [36,47]. NAMs are markedly increased in NASH, which is induced by a high-fat diet [29,30**]. The loss of TREM2 exacerbates hepatic lipid accumulation and inflammation [48], suggesting a protective function of NAMs against hepatic injury triggered by lipid overload. Collectively, TREM2+ macrophages appear to be protective against chronic inflammation. However, the exact function of TREM2+ macrophages in chronic inflammation should be elucidated using a conditional loss-of-function approach.

**COMMON FEATURES OF TWO TYPES OF LIPID-ASSOCIATED MACROPHAGES IN CASES OF OBESITY AND ATHEROSCLEROSIS**

Obesity and atherosclerosis have common features of disease progression. Both diseases are induced by an imbalance in energy intake and expenditure, leading to hyperlipidaemia and chronic inflammation in various tissues. Obesity is strongly associated with adipose tissue inflammation, leading to insulin resistance and type 2 diabetes mellitus [49]. Macrophages are crucial effector cells involved in obesity-induced adipose inflammation and insulin resistance [50]. The Ido Amit group described a novel and highly conserved TREM2+ macrophages, named LAMs, in the adipose tissue during obesity [4**]. LAMs possess lipid droplets and express the lipid receptor TREM2 and lipid metabolism-related genes residing in crown-like structures surrounding adipocytes of obese mice. However, they are not present in the normal state. Previously, we demonstrated that PLAMs also show enhanced expression of TREM2 and other lipid metabolism-related genes. Therefore, we compared the gene expression profiles of LAMs and PLAMs. These two macrophages showed highly conserved gene expressions, including *Lipa*, *Cstl*, *Fabp4*, *Fabp5*, *Lgals3*, *Cd9* and *Cd36*. Next, we defined the enriched genes in LAMs (*n* = 65, log2FC >2.5, vs. normal adipose tissue macrophages) and analysed their expressions in our scRNA-seq data from atherosclerotic aortas. We found that the enriched genes in LAMs were highly correlated with those enriched in PLAMs (Fig. 1). In particular, these two LAMs share a common feature of gene expression related to phagocytosis, endocytosis, lysosomes, lipid metabolism, peroxisome proliferator-activated receptor gamma and oxidative phosphorylation (Fig. 1). These results suggest that LAMs present in cases of obesity and atherosclerosis have similar functions in a lipid-enriched inflammatory milieu.

**POSSIBLE ROLE OF PLAQUE LIPID-ASSOCIATED MACROPHAGES IN THE PATHOGENESIS OF ATHEROSCLEROSIS**

In a previous study, *Trem2*−/− mice showed increased adipose hypertrophy, insulin resistance and hyperlipidaemia. Mice transplanted with *Trem2*−/− bone marrow also showed the same phenotypes, suggesting that TREM2 expressing LAMs may be responsible for the protective effect of TREM2 against metabolic inflammation [4**]. Considering the similarities in the gene expressions between LAMs and PLAMs, PLAMs can be expected to suppress plaque inflammation by eliminating apoptotic cell debris and modified lipids. Indeed, myeloid LXR deficiency accelerated atherosclerosis and decreased the number of plaque TREM2+ foamy macrophages, that is PLAMs, with decreased expression of TREM2 downstream genes related to cholesterol transport and metabolism, whereas inflammatory gene expressions were increased in nonfoamy and foamy macrophages [51**]. These results suggest that PLAMs may increase the expression of genes related to lipid metabolism via a collaborative interaction between TREM2 and LXR to cope with the lipid-rich atherosclerotic milieu.

**CONCLUSION**

The phenotypes and functions of macrophages in various disease settings have been widely investigated using single-cell transcriptome analysis, and the heterogeneity and function of macrophages in atherosclerosis have been elucidated. PLAMs commonly express genes related to phagocytosis, lysosomal
activity and lipid metabolism and are expected to play a role in maintaining homeostasis in response to tissue injury. However, to understand the exact function of PLAMs and develop novel therapeutic regimens, extensive and comprehensive studies are required. For example, whether PLAMs originate from initial inflammatory macrophages or from a specific population of monocytes/macrophages and how macrophages gain the PLAM phenotype in atherosclerotic plaques need to be determined. Moreover, the exact function of TREM2 in the pathogenesis of atherosclerosis remains unclear. A conditional loss-of-function approach using macrophages is required to understand the exact role of PLAMs in the pathogenesis of atherosclerosis.

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Conflicts of interest
There are no conflicts of interest.

FIGURE 1. Enriched genes and pathways in Trem2 hi macrophages present in cases of atherosclerosis and obesity. Top: single-cell RNA sequencing data from Kim et al. [3] are re-visualized using UMAP clustering. Bottom: The top 65 LAM genes from Jaitin et al. [4] are highly upregulated in PLAMs (Cluster 7). FC, fold change; LAM, lipid-associated macrophages; PLAM, plaque LAM; PPAR, peroxisome proliferator-activated receptor; UMAP, Uniform manifold approximation and projection.
This study reported Trem2-expressing LAMs from obese adipose tissue in mice identify the standard features and contributing factors of macrophages affecting this study is helpful to obtain the current understanding about the function of TREM2 and its therapeutic application. This review study summarized the of TREM2 and its therapeutic application. This study demonstrated that LXR activation is important to induce the Trem2 gene and thereby facilitates uptake of amyloid-beta by microglia. Neon 2016; 91:528–540.

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This study demonstrated that LXR activation is important to induce the Trem2 gene expression programme leading to suppression of inflammation in the foamy macrophages of atherosclerotic plaques.

This study is a meta-analysis using new algorithms (AtheroSpectrum) to identify the standard features and contributing factors of macrophages affecting the cause of atherosclerosis in mouse models and human patients.