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Letter by Cochain et al Regarding Article, "Transcriptome Analysis Reveals Nonfoamy Rather Than Foamy Plaque Macrophages Are Proinflammatory in Atherosclerotic Murine Models"

To the Editor:

With great interest we have read the report by Kim et al[1] suggesting that nonfoamy rather than foamy macrophages express proinflammatory cytokines in murine atherosclerosis. In this article, the authors developed a new method for staining and sorting of lipid-laden foamy macrophages from murine atherosclerotic aortas of *Ldlr*$^{-/-}$ and *Apoe*$^{-/-}$ mice and characterized this cell population by bulk RNA sequencing (RNA-seq) and single-cell RNA-seq. These analyses demonstrated that the expression of prototypical inflammatory cytokines (eg, *Il1b*, *Cxcl2*, *Ccl2*, and *Tnf*) was mostly confined to nonfoamy macrophages, whereas foamy macrophages expressed low levels of inflammatory cytokines and were enriched in genes involved in, for example, oxidative phosphorylation or cholesterol metabolism. These novel and surprising findings are, from our point of view, of critical importance for our understanding of macrophage biology in vascular inflammation and atherosclerosis.

In a recent study,[2] we have also performed single-cell RNA-seq of total leukocytes from the healthy and atherosclerotic aorta of *Ldlr*$^{-/-}$ mice, which led us to identify several atherosclerosis-associated macrophage subsets. One subset showed a discrete and, to our knowledge, previously undescribed gene expression profile, characterized by high expression of genes, such as *Trem2*, *Cd9*, *Ctsd*, or *Spp1*, low expression of inflammatory cytokines, and putative biological functions, such as organic substance and cellular catabolic processes, lipid metabolic processes, or regulation of cholesterol efflux. Based on *Trem2* being the most significantly enriched gene in this cell subset in our primary analysis, we termed this macrophage subset TREM2^{hi} macrophage. In our analysis, expression of prototypical proinflammatory cytokines (eg, *Il1b*, *Cxcl2*, *Ccl2*, and *Tnf*) by macrophages was mostly confined to a subset we termed inflammatory macrophages. A similar single-cell transcriptomic profile was observed in macrophages from atherosclerotic *Apoe*$^{-/-}$ aortas.[2,3]

On reading the report by Kim et al,[1] it quickly struck us that the transcriptomics profile associated with foamy macrophages (Kim et al) was remarkably similar to that of TREM2^{hi} macrophages, both showing an enrichment in similar genes (eg, *Itgax*, *Lgals3*, *Trem2*, and others) and in similar putative functions. To test the hypothesis that the cell subset we termed TREM2^{hi} macrophages actually corresponds to foamy macrophages described by Kim et al, we evaluated the expression of genes enriched in foamy and nonfoamy macrophages from *Apoe*$^{-/-}$ mice (as determined in bulk RNA-seq experiments by Kim et al) in our macrophage populations from aortas of *Ldlr*$^{-/-}$ mice fed a high-fat diet for 11 or 20 weeks or *Apoe*$^{-/-}$ mice after 12 weeks of high-fat diet feeding[3] (obtained from single-cell RNA-seq of CD45^{+} leukocytes). In each case, expression of foamy macrophage genes was enriched in TREM2^{hi} macrophages, whereas nonfoamy macrophage genes were enriched in inflammatory macrophages (Online Figure I).

In the reverse approach, we furthermore evaluated the expression of TREM2^{hi} macrophage marker genes (39 genes characterizing TREM2^{hi} macrophages versus other aortic macrophage subsets in our single-cell differential expression analysis) in the single-cell RNA-seq dataset from Kim et al. In total CD45^{+} aortic cells from *Ldlr*$^{-/-}$ mice, the cell cluster enriched in these TREM2^{hi} macrophage marker genes corresponded to putative foamy macrophages (equivalent to cluster 4 in the analyses by Kim et al). Moreover, isolated lipid-laden foamy macrophages of leukocyte origin (expressing *Ptprc*, encoding CD45) from *Apoe*$^{-/-}$ aortas showed enrichment in TREM2^{hi} macrophage genes, in contrast to foam cells of nonhematopoietic origin (Online Figure II).

Altogether, this analysis clearly suggests that the transcriptomic profile of the population we termed TREM2^{hi} macrophages indeed corresponds to the foamy macrophages described by Kim et al. The study by Kim et al thus provides a critical new layer of information by associating this gene expression profile to a cellular functional state.

With the advent of relatively affordable methods to perform single-cell RNA-seq on thousands of cells and user-friendly analysis pipelines, it is to be anticipated that single-cell RNA-seq will become a method of choice for gene expression analysis in experimental models of atherosclerosis and other cardiovascular diseases, in particular to further decipher immune cell phenotype and function. To avoid creating confusion in the literature, however, it will be crucial that researchers carefully compare their transcriptomic datasets to existing ones and establish (or exclude) potential equivalencies between cell populations. Taking this into consideration, it will now be interesting to further build on these findings to deepen our understanding of macrophage biology in atherosclerosis but possibly also other diseases in future studies.

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Disclosures

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