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INVITED SPOTLIGHT REVIEW

Heterogeneity of immune cells in human atherosclerosis revealed by scRNA-Seq

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Abstract Immune cells in atherosclerosis include T, B, natural killer (NK) and NKT cells, macrophages, monocytes, dendritic cells (DCs), neutrophils, and mast cells. Advances in single-cell RNA sequencing (sRNA-Seq) have refined our understanding of immune cell subsets. Four recent studies have used scRNA-Seq of immune cells in human atherosclerotic lesions and peripheral blood mononuclear cells (PBMCs), some including cell surface phenotypes revealed by oligonucleotide-tagged antibodies, which confirmed known and identified new immune cell subsets and identified genes significantly up-regulated in PBMCs from HIV^+ subjects with atherosclerosis compared to PBMCs from matched HIV^+ subjects without atherosclerosis. The ability of scRNA-Seq to identify cell types is greatly augmented by adding cell surface phenotype using antibody sequencing. In this review, we summarize the latest data obtained by scRNA-Seq on plaques and human PBMCs in human subjects with atherosclerosis.

Keywords Atherosclerosis • scRNA-Seq • Transcriptomes • Antibodies • Human

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1. Immune cells in cardiovascular disease

Cardiovascular diseases (CVDs) remain the leading cause of death worldwide, just ahead of cancer and COVID-19. CVD is a broad term that includes diseases like stroke, myocardial infarction (MI), or peripheral artery disease. Here, we focus on atherosclerosis, which is responsible for most CVDs. Atherosclerosis is a chronic inflammatory disease of the arteries that is associated with elevated lipids and specifically elevated low-density lipoprotein (LDL) cholesterol. Although hypercholesterolaemia is necessary for the initiation of the disease, immune mechanisms play a key role in lesion development, progression, and vulnerability.[1] Vulnerability refers to the propensity of a lesion to rupture or erode, which correlates with plaque cap thickness and immune cell content.[2]

In 2017, the Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) provided a first proof of concept that the risk of atherosclerotic cardiovascular events can be reduced by dampening the inflammatory response.[3] In CANTOS, a monoclonal antibody targeting interleukin (IL)-1β (canakinumab) was given to patients with a history of MI and a residual inflammatory risk, defined as high-sensitivity C-reactive protein (hsCRP) ≥2 mg/L. Patients receiving canakinumab had a 15% reduction in the relative risk of experiencing an atherosclerotic cardiovascular event and concomitant dramatic reductions in blood hsCRP and IL-6. Importantly, blood lipids, including LDL cholesterol, were unaffected by the anti-IL-1β treatment, stressing the specific anti-inflammatory effect of canakinumab. This study has paved the way for a search for more efficient, targeted and safer treatments of the residual inflammatory risk in atherosclerosis.

2. Heterogeneity of immune cells in atherosclerosis

The types of innate and adaptive immune cells found in atherosclerotic lesions were recently reviewed.[4–9] Investigations into the heterogeneity of immune cells in atherosclerotic lesions began with immunostaining studies in the 1980's,[10] which at the time allowed staining for about two markers. Immunostaining in tissues has recently been refined to resolve about 16 markers.[11] Flow cytometry was introduced into atherosclerosis research in 2006,[12] expanding the range of markers. The next

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technological advance was mass cytometry (CyTOF),[13,14] reaching about 40 markers. Here, we focus on the highest resolution methods available today: single-cell RNA sequencing (scRNA-Seq; up to 3000 transcripts per cell or even more depending on sequencing depth and scRNA-Seq method) with antibody sequencing (up to 200 antibodies) and T- and B-cell receptor sequencing (both α and β, heavy and light chains).[15] scRNA-Seq data obtained from immune cells of mouse atherosclerotic aortas was recently reviewed in a meta-analysis.[16] This review is focused on the most recent data obtained by scRNA-Seq and antibody sequencing (also known as CITE-Seq or REAP-Seq) of human atherosclerotic lesions.

3. scRNA-Seq of human atherosclerotic lesions

scRNA-Seq data of immune cells in human plaques and/or human peripheral blood mononuclear cells (PBMCs) from individuals with atherosclerosis are contained in four recent publications.[17–20] *Figure 1* illustrates an integration of mouse[14,21–25] and human[17,18] myeloid cells in atherosclerotic arteries projected into the same UMAP. The main goal of three of the four studies[17,18,20] was to link scRNA-Seq features to cardiovascular events. One study[20] was focused on the discovery of heterogeneous leukocyte subsets and their transcriptomes. Three studies focus on immune cell subsets, whereas Wirka *et al.*[18] is mainly covering smooth muscle cell (SMC) phenotypes. The technical aspects of the studies are summarized in *Table 1*. The technical challenges of scRNA-Seq of immune cells isolated from blood vessels including enzymatic digestion, doublets, dead cells, cell stress, batch effects, and others were recently reviewed.[26–30] Enzymatic tissue digestion and mechanical tissue dissociation may lead to uneven loss of certain and not other cell types, which might explain the variability observed across the studies. T cells, which are small and robust, can survive the isolation procedure better than macrophages or dendritic cells, which are large and fragile. The cellular stress response during enzymatic processing can induce artefacts[26] that affect the outcome of the study. scRNA-Seq yields mainly high expressed genes and systematically misses genes with low expression. Because of differences in isolation procedures, sample types and sample collection, as well as experimental variations, a direct comparison between the studies is challenging. Key marker genes for major immune cell subsets in human atherosclerotic arteries are shown in *Figure 2*.

The foundational paper of human plaque scRNA-Seq[17] analysed leukocytes from human plaques obtained by carotid endarterectomy and matched PBMCs. Endarterectomy specimens contain plaque, fibrous cap and sometimes part of the media of the carotid, but not the adventitia. A total of 1654 PBMCs and 254 cells from the endarterectomy specimen from the same patient were analysed by 10× Genomics 3′ scRNA-Seq combined with surface phenotype defined by 21 mAbs. An additional four asymptomatic and two symptomatic endarterectomy specimen were analysed by scRNA-Seq without surface phenotype. Doublets were partially removed by rejecting droplets that contained barcodes from both PBMCs and plaque. However, this method cannot detect PBMC–PBMC or plaque–plaque doublets. Therefore, these doublets remain in the data set, yielding mixed cell transcriptomes. Current bioinformatics tools based on multiple hashtags or synthetic transcriptomes cannot completely eliminate doublets.

The authors found 16 T-cell clusters (*Table 2*) in both plaque and blood. Unexpectedly, $CD8^+$ T cells were enriched in plaque (46%) compared to blood (10%). Blood T-cell transcriptomes were enriched for resting CD4 T cells and expressed genes inhibiting T-cell functions (*KLF2* and *TXNIP*). Plaque T cells expressed transcripts associated with T-cell activation (*NFATC2, FYN, ZAP70*), cytotoxicity (*GZMA, GZMK*), and T-cell exhaustion (*EOMES, PDCD1, LAG3*). A sub-analysis showed that plaque $CD4^+$ T cells were in an activated pro-inflammatory state (Th1 functions, *KLRD1, KLRC1, CXCR3, STAT3, IFNGR1, HLA-B*; chemotaxis, *CCL5, CCL4, CXCR6*).

Although antibody sequencing was used in only one plaque sample, its power was revealed by identifying five distinct macrophage clusters. Cluster 1 expressed genes involved in macrophage activation (*HLA-DRA* and *CD74*). Cluster 2 was highly inflammatory, expressing genes involved in inflammatory responses (*CYBA, LYZ, S100A9/8, AIF1*), toll-like receptor binding (*S100A9/8*), and oxidoreductase activities (*CYBA*) and the metalloprotease inhibitor *TIMP1*. Cluster 3 uniquely up-regulated genes involved in pro-inflammatory responses (*JUNB, NFKBIA*) and highly

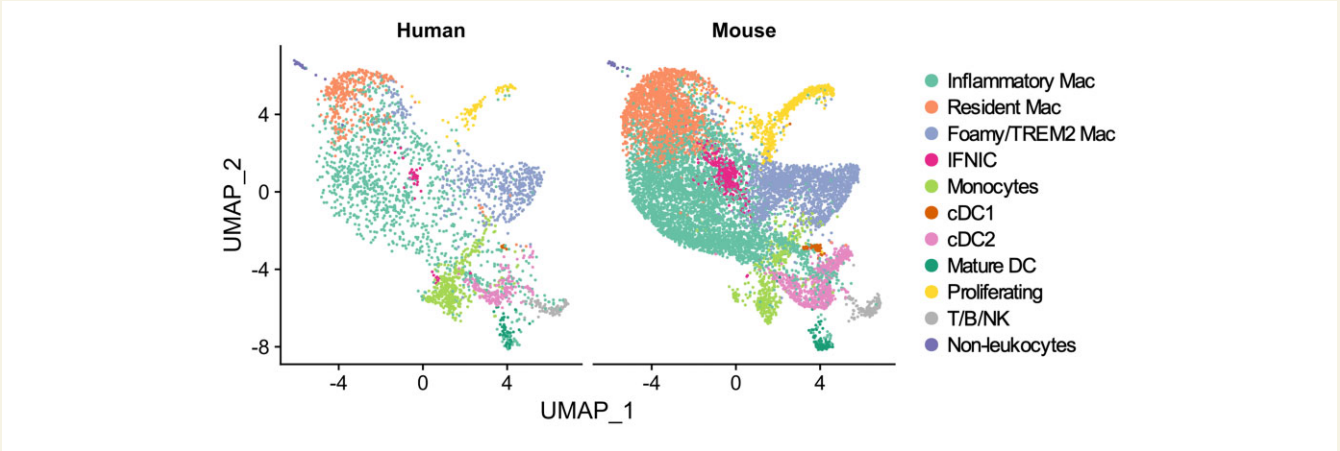

Figure 1 Human and mouse myeloid cells in atherosclerotic arteries. scRNA-Seq data from mouse[14,21–25] (right) and human[17,18] (left) myeloid cells were projected onto the same UMAP and then separated by species. Inflammatory (green), resident (orange), foamy (pale purple), and interferon (IFNIC, pink) macrophages were found in both mouse and human arteries, as were monocytes (light green), mature DCs (dark green), cDC1 (brown), and cDC2 (pale pink). Some contaminating T, B, and NK cells (grey), some proliferating cells (yellow), and a handful non-leukocytes (purple) were also found. *N* = 2890 human and 10 849 mouse cells, respectively. cDC, conventional dendritic cells; Mac, macrophages.

Table 1 Technical information for each of the human scRNA-Seq studies

Study → Technicalities ↓	Fernandez[17]	Wirka[18]	Depuydt[19]	Vallejo[20]
Source of material	Endarterectomy/PBMCs	Coronary arteries	Endarterectomy	PBMCs
Digestion	37°C for 1 h in 10 mL DMEM containing 10% FBS; collagenase type IV at a final concentration of 1 mg mL^{-1}; and DNase, hyaluronidase, collagenase type XI and collagenase type II, each at a final concentration of 0.3 mg mL^{-1}	RNase A and proteinase K. Enzymatic dissociation cocktail (10.4 U mL^{-1} Liberase and 8 U mL^{-1} elastase in 1 ml HBSS)	The tissue was digested in RPMI 1640 containing 2.5 mg/mL Collagenase IV, 0.25 mg/mL DNAse I, 2.5 mg/mL Human Albumin Fraction V and 1 mM Flavopiridol at 37°C for 30 min	None
Initial total number (*N*) of cells	7169	3707	NA	54 078
(*N*) of cells analysed after QC	3563	3643	3282	41 611
Platform	10× genomics chromium	10× genomics chromium	10× genomics chromium	BD rhapsody
Bioinformatic pipeline	Cell ranger	Cell ranger	Cell ranger	Seven bridges genomics
QC	Cell ranger	Cell ranger	Cell ranger ATAC pipeline	Seven bridges
Doublet removal procedure	Exclusion of overlapping barcodes	Discarding cells with more than 3500 genes (1.7% doublets)	Only cells between 500 and 10 000 genes and genes expressed in at least three cells	Overlapping barcodes and Doublet Finder (based on synthetic transcriptomes)
Batch effect reduction	Mutual nearest neighbours	NA	NA	All samples were hashtagged and multiplexed. Minimal batch effect verified
Viability	NA	Cells with less than 500 genes and genes expressed in fewer than five cells were excluded	Only cells between 500 and 10 000 genes and genes expressed in at least three cells	Cell viability 88 ± 5%
(*N*) reads/cell	111 670 mean reads for scRNA-Seq 50 701 mean reads for Cite-Seq	Total 516 440 244 paired-end reads Mapped 512 644 923 paired-end reads After removal of duplicates, 43 727 785 paired-end reads	NA	Total 60 600 reads/cell AbSeq: 40 000 reads/cell mRNA: 20 000 reads/cell Sample tags: 600 reads/cell
% mitochondrial reads	Ribosomal and mitochondrial genes were dropped from the gene expression analysis	Cells with more than 7.5% of mitochondrial genes were excluded	Mitochondrial and ribosomal genes, MALAT1, KCNQ1OT1, UGDH-AS1, and EEF1A	No mitochondrial genes
Surface phenotypes (*N*)	21	11	7	40
Transcriptomes (*N*)	NA	NA	3876 DEG	41 611
(*N*) of immune cell clusters	27 endarterectomies 19 PBMCs	7	16	58
scRNA-Seq significance	Immune cells in the plaque and their different activation states. T cells in plaque are more activated, differentiated and exhausted compared to blood	Identification of Tcf21 as phenotypic modulator associated with protection from coronary artery disease	Intercellular communication in human atherosclerotic plaque	New inflammatory signatures in PBMCs of women with HIV or CVD or both, some receiving cholesterol-lowering drugs

DEG, differentially expressed genes; *N*, number; NA, not available; QC, quality control.

expressed *MALAT1*. These clusters may correspond to the inflammatory macrophages identified in mouse atherosclerosis (*Figure 1*).[16] Cluster 5 expressed genes involved in cholesterol uptake and metabolism (*APOC1, APOE*) and lipid accumulation (*PLIN2*), similar to TREM2 foam cells identified in mouse atherosclerosis.[16] These macrophages showed reduced pro-inflammatory signalling (*IL-1, IFN*), consistent with the anti-inflammatory nature of foam cells.[31] A total of 46 samples (29 from asymptomatic patients and 17 from symptomatic patients) were

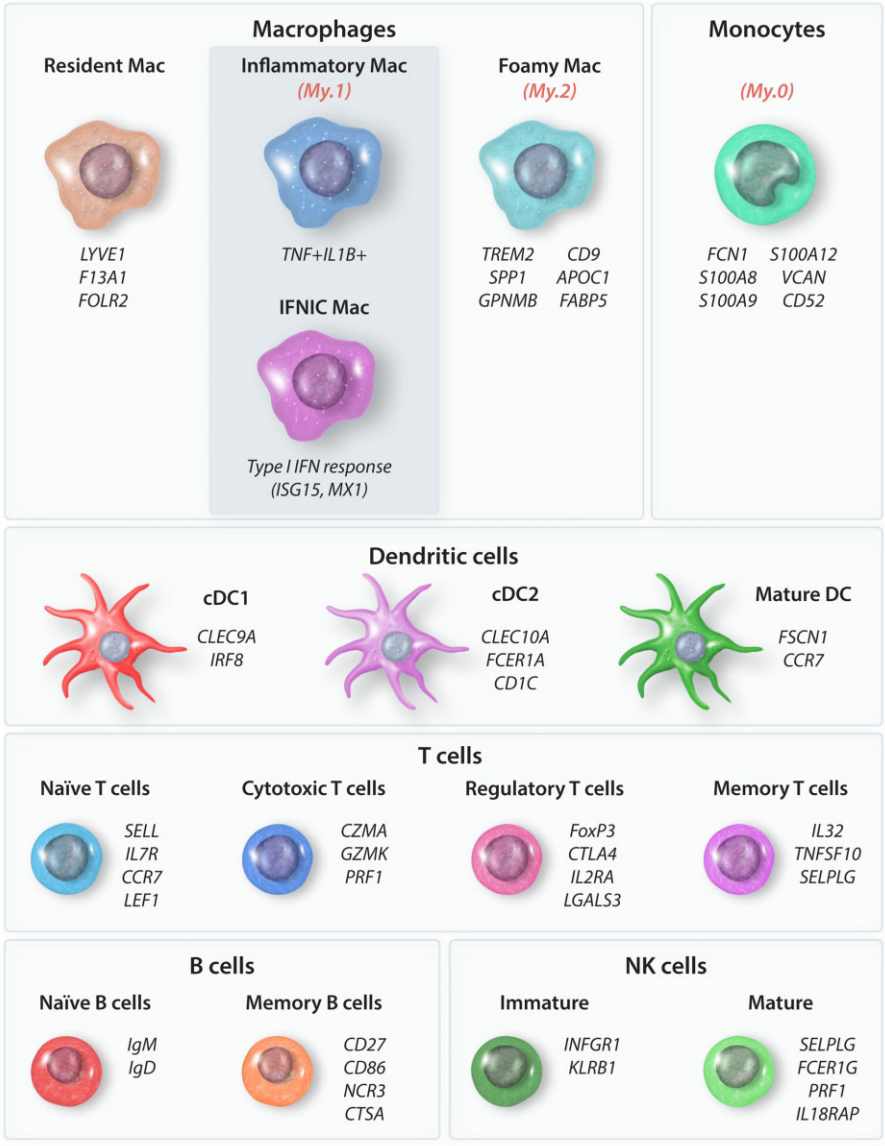

Figure 2 Key marker genes for major immune cell subsets in human atherosclerotic arteries. Resident and foamy macrophages were resolved in Depuydt *et al.*[19] My.0, My.1, and My.2 indicate the annotations used in Depuydt *et al.*[19] Genes from each of the T-cell subsets were obtained from Depuydt *et al.*,[19] Fernandez *et al.*,[17] and/or Vallejo *et al.*[20] B and NK genes were resolved in Vallejo *et al.*[20] cDC, conventional dendritic cells; Mac, macrophages.

subjected to mass cytometry,[13,14] which yielded only 2 distinguishable macrophage clusters. Although CyTOF can detect intracellular cytokines or transcription factors,[14] it provides no information on transcriptomes. The number of markers used and picked in CyTOF, even though informative, is smaller than in scRNA-Seq, which leads to lower resolution of immune cell subsets. Vallejo *et al.*[20] used 40 oligonucleotide-tagged antibodies on 32 samples, thus combining accurate cell surface phenotyping similar to CyTOF with transcriptomes.

Wirka *et al.*[18] used cells dissociated from human coronary arteries from explanted hearts. Unlike endarterectomy specimens, these samples include adventitial material. The study was focused on SMCs and fibromyocytes, but here we only review the immune cell subsets found [macrophages, T, B, and natural killer (NK) cells]. The authors used 10× Genomics using 150 bp paired-end reads. Cells with less than 500 genes and genes expressed in fewer than five cells were excluded from analysis. Doublets were reduced by discarding cells with more than 3500 genes, but not methods like DoubletFinder,[32,33] which are better suited for doublet removal. Cells with mitochondrial gene content above 7.5% were considered non-viable. The authors identified seven immune cell clusters: macrophages expressing *RNASE1, C1QA, C1QC, C1QB*, and *CD14*; T cells expressing *IL32, TRAC, IL7R, CCL5*, and *CD3D*; B cells expressing *CD79A, CD37, MS4A1, LTB, CD52*, and IgM plasma cells expressing *IGHM, JCHAIN, IGLC3, IGHV3-73, MZB1*; IgG plasma cells expressing *IGHG2, IGHGP, MZB1, DERL3*; and *PIM2*, NK cells expressing *NKG7, GNLY, PRF1, GZMB, CCL5*; and mast cells expressing *TPSAB1, CPA3, C1orf186, SLC18A2, MS4A2*. Quantitative analysis (cells per cluster) was not provided. PBMCs were not analysed.

Table 2 Immune cell subsets detected by human scRNA-Seq

Immune subsets → Study ↓	T cells	B/plasma	NK cells	Macrophages	Monocytes	Other
Fernandez[17] Endarterectomy	16	2	1	5	0	2 DC 1 NKT
Fernandez[17] PBMCs	16	1	1	0	1	NA
Wirka[18] Coronary	1	3	1	1	0	1 mast
Depuydt[19] Endarterectomy	8	1	0	5	0	1 mast 1 DC
Vallejo[20] PBMCs	30	6	6	0	16	DC, $\gamma\delta$ T cells

DC, dendritic cell; NA, not available.

The third publication on human plaque scRNA-Seq appeared in December of 2020.[19] Plaques were from carotid endarterectomies from 14 males and 4 females. Six each were classified by histology as fibrous, fibro-atheromatous, and atheromatous, respectively. Depuydt *et al.*[19] analysed three matched samples by immunohistochemistry using CD3, CD68, CD34, and anti-SMC antibodies. Within the confines of these four antibodies, the immunofluorescence data are consistent with the scRNA-Seq data. Two samples were analysed by single cell-single well CEL-Seq2, sequenced as 2 × 75 bp paired-end reads. Viable cells were sorted one cell per well into 384-well plates. Reads were filtered by mitochondrial and ribosomal genes (*MALAT1, KCNQ1OT1, UGDH-AS1*, and *EEF1A*) to remove dead cells. *MALAT1* is a long non-coding RNA that has been implicated in atherosclerosis[34] and it is highly associated with the development and progression of human cancers.[35,36] In mice, expression of *MALAT1* is inversely correlated with cell health; dead/dying cells have higher expression of *MALAT1*.[37] These findings explain why the authors included this gene to remove dead/dying cells. Only cells between 500 and 10 000 genes and genes expressed in at least three cells were used for further analysis. Single cell-single well technology provides much deeper transcriptomes than 10× Genomics but is much more expensive and yields much fewer cells. Three endarterectomy samples were subjected to epigenetic analysis using the assay for transposable accessible chromatin (ATAC-Seq).[38] Unbiased clustering was performed on transcriptomes from 3282 cells without removing cells that may have been stressed by the digestion process. The authors focused on genes that are specific for the different cell types in plaques or are genetically associated to coronary artery disease. Of the 11 immune cell populations identified, 5 were lymphocyte clusters, 5 were myeloid and one could not be identified. A total of seven different antibodies (CD45-PECy7, CD3-BV421, CD4-PETR, CD28-BV650, Granzyme B-PE, TruStain Fcx, and Fixable Viability Dye-eFluor 780) were used for sorting the cells into the wells.

The authors found five subsets of $CD4^+$ T cells (*Figure 2*). Two were cytotoxic, one expressing *PRF1, GZMA*, and *GZMK*, but little *CD28* transcript, suggesting these cells may be $CD4^+$ $CD28^{null}$[39] and the other expressed *GZMA, GZMK*, and *CD28*. One subset of naïve $CD4^+$ T cells expressed *IL7R, LEF1*, and *SELL*. Regulatory $CD4^+$ T cells were identified by *FoxP3, IL2RA*, and *CTLA4*; and a central $CD4^+$ T memory cell expressed *LEF1, IL7R*, and *SELL*. Among the three subsets of $CD8^+$ cells, they found $GZMK^+$ effector memory $CD8^+$ T cells expressing *GZMK, GZMA*, and *CD69*, one cluster of terminally differentiated cytotoxic $CD8^+$ T cells expressing *GZMB, TBX21, NKG7, GNLY, ZNF683*, and *CX3CR1* and lacking *CD69*, and one central memory $CD8^+$ T-cell cluster expressing *LEF1, SELL, IL7R*, and *LTB*. The exhausted phenotype reported by Fernandez *et al.*[17] was not observed. Fernandez *et al.*[17] reported exhausted $CD8^+$ T cells based on increased PD-1, EOMES and LAG-3 expression.[40,41] Depuydt *et al.*[19] show elevated levels of CD69 in their $CD8^+$ T-cell clusters, suggesting increased TCR activation rather than exhaustion.

In the initial clustering, Depuydt *et al.*[19] found five clusters of myeloid cells: a mast cell population identified by *HDC, KIT, CMA1, TPSAB1* and four clusters that expressed *CD14* and *CD68*. These four clusters were further reclustered, obtaining five distinct macrophage phenotypes. Two inflammatory macrophage subsets expressed *IL1B, CASP1, CASP4, KLF4*, and *KLF4, IL1B, TLR4, ABCA1, TNF, IL18, CD9*, respectively, and were named $IL1B^+$ and TNF^+ inflammatory macrophages, respectively. One subset contained $ABCG1^+$ foamy macrophages expressing *ABCA1, ABCG1, MMP9, OLR1, TREM2, CD9, ACTA2, LGALS3, CD68, IL18*, and *CD9*. A $CD1c^+$ dendritic cell subset expressed *CD1c, CLEC10A*, and *FCER1A*. The fifth cluster of myeloid cells contained both T-cell (*CD3E, GNLY, FOXP3, CD2*) and myeloid (*CD14, CD68, KIT*) markers, suggesting that this cluster may be composed of doublets between monocytes and T cells[42] (*Figure 2*).

Like in the studies of Fernandez[17] and Winkels *et al.*,[14] T cells were the majority (52%) of analysed cells, compared with only 19% myeloid cells. Meta-analysis of the Winkels mouse dataset[14] together with eight other mouse datasets[16] revealed that the enrichment of T cells was due to loss of myeloid cells, likely caused by the digestion procedure. Large branched macrophages are less robust than small round lymphocytes.[26] Thus, the low representation of myeloid cells by Fernandez[17] and Depuydt *et al.*[19] may, in part, be caused by preferential loss of macrophages. In addition, endarterectomy specimen lack most of the media and all of the adventitia, where many macrophages reside.

The most recent scRNA-Seq study on atherosclerosis was conducted on PBMC samples from 31 female subjects, 16 of whom had subclinical atherosclerosis as defined by carotid ultrasound.[20] Twenty-four of the 31 women studied lived with HIV, with most having undetectable viral loads. The study used BD Rhapsody for 485 genes and 40 antibodies. In total, 32 000 cell transcriptomes were obtained. No plaque samples were analysed. The authors identified 58 different subsets among $CD4^+$ T cells (16 identified clusters), $CD8^+$ T cells (14 identified clusters), B cells (6 clusters), NK cells (6 clusters), and monocytes (8 classical, 2 non-classical and 5 intermediate monocyte clusters). To test for changes with CVD, they analysed genes significantly different between subjects with and without CVD. All these subjects were HIV^+.

Within T cells, in two of the effector memory CD4$^+$ T clusters, *IL-32* was highly significantly increased by CVD. IL-32 is an inflammatory cytokine that is known to be important in CVD.[43,44] In the naïve CD4$^+$ T-cell cluster, IL-32, L-selectin (*SELL*), PSGL-1 (*SELPLG*), and *CCR7* were also highly significantly increased in participants with CVD. In addition to *SELL* and *SELPLG*, one of the effector memory CD4$^+$ T clusters showed strong up-regulation of TNFSF10 (*TRAIL*). In the terminally differentiated memory (EMRA) CD8$^+$ T cluster, *IL32* was high in women with CVD. This was true even in the naïve CD8$^+$ T-cell cluster. In one EMRA CD8$^+$ T-cell cluster, *CD52, TRAC*, and *HOPX* were significantly up-regulated by CVD, as were several killer cell lectin receptors (*KLRC4, KLRD1, KLRG1*, and *KLRK1*). A second cluster of EMRA CD8$^+$ cells showed higher *CD52, IL32, CD160*, and *CCL5* in participants with CVD. The chemokine CCL5 and its primary receptor CCR5 are involved in the development of atherosclerosis and MI.[45,46] CCL5 encodes the chemokine RANTES, known to be important in atherosclerosis.[47] In fact, the manipulation of CCL5 or its receptor has shown beneficial effects in animal models reducing neointima formation and macrophage infiltration[48] as well as atherosclerotic plaque formation.[49] CCL5 has also been used as a possible biomarker for CVD in several studies.[50,51] Lastly, in the effector memory CD8$^+$ T cluster, CVD was associated with significantly increased *IL32, TRAC, HOPX, CCL5* and the killer lectin receptors *KLRK1, KLRC4, KLRD1*.

In one classical monocyte (CM) cluster, CVD was associated with significantly increased *CCL4, SLC2A3, SOD2*, and *SELPLG*. In another CM cluster, *TNF, DUSP1*, and *2* were highly associated with CVD, as were *TNFSF10* (*TRAIL*), *TNFSF13* (*APRIL*), and *TNFSF13B* (*BAFF*), important B-cell regulators. *CCL3, CCL4, IL1B*, and *DUSP2*, known to be relevant in atherosclerosis, were highly up-regulated in a third CM subset in CVD$^+$ participants. The Toll-like receptor *TLR2*, which is known to be involved in atherosclerosis, was also up-regulated in this same CM cluster. In one of the intermediate monocyte clusters, *CCL3, CCL4, TNF, IL1B*, and *DUSP2* were associated with CVD.

4. Concluding remarks

Although scRNA-Seq without antibodies is able of detect immune cell subsets, many of the subsets are not well resolved. For example, it remains challenging to distinguish CD4 from CD8 T cells using scRNA-Seq transcriptomes without surface markers. scRNA-Seq without surface phenotype fails to identify some immune cell types based just on mRNA information. This has led to much frustration in this field because the expression of genes encoding even major cell surface markers are not detected. The addition of cell surface phenotype information greatly improves the immune cell identification, because immune cell types have been defined by surface phenotype based on 30 years of flow cytometry. The power of this technique is illustrated in Fernandez *et al.*[17] (21 oligonucleotide-tagged antibodies) and Vallejo *et al.*[20] (40 oligonucleotide-tagged antibodies). Now panels up to 200 antibodies are available for human and mouse cells, which, in combination with deeper transcriptomes from scRNA-Seq, will increase the resolution of immune cell subsets and increase chances to discover new cell subsets. To develop atherosclerosis-specific gene signatures, healthy controls must be included, which is not possible in studies based on endarterectomy specimens but can be done with PBMCs (Vallejo *et al.*[20]) and non-atherosclerotic coronary arteries from explanted hearts (Wirka *et al.*[18]). We can expect that scRNA-Seq combined with cell surface phenotypes will increase the resolution and quality of the immune cell atlas in human atherosclerosis.

Authors' contributions

J.V. and K.L. wrote and edited the manuscript. J.V. compiled the tables. J.V., C.C., and A.Z. drew the figures. J.V., C.C., A.Z., and K.L. revised the manuscript.

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