Review

Myeloid Cell Diversity and Impact of Metabolic Cues during Atherosclerosis

Alexandre Gallerand †, Marion I. Stunault †, Johanna Merlin †, Rodolphe R. Guinamard, Laurent Yvan-Charvet, Stoyan Ivanov *

Mediterranean center of molecular medicine (C3M)–Université Côte d'Azur–INSERM U1065, Team 13, Nice, 06200, France

† These authors contributed equally to this work.

* Correspondence: Stoyan Ivanov, Email: Stoyan.ivanov@unice.fr.

ABSTRACT

Myeloid cells are key contributors to tissue, immune and metabolic homeostasis and their alteration fuels inflammation and associated disorders such as atherosclerosis. Conversely, in a classical chicken-and-egg situation, systemic and local metabolism, together with receptor-mediated activation, regulate intracellular metabolism and reprogram myeloid cell functions. Those regulatory loops are notable during the development of atherosclerotic lesions. Therefore, understanding the intricate metabolic mechanisms regulating myeloid cell biology could lead to innovative approaches to prevent and treat cardiovascular diseases. In this review, we will attempt to summarize the different metabolic factors regulating myeloid cell homeostasis and contribution to atherosclerosis, the most frequent cardiovascular disease.

KEYWORDS: macrophage; monocyte; dendritic cell; metabolism; atherosclerosis

INTRODUCTION

Atherosclerosis is a major vascular disease that continuously spreads worldwide. Atherosclerosis contributes to cardiovascular disease (CVD)-related deaths, estimated to account for more than 17 million deaths per year worldwide, making this pathology a major public health issue. Atherosclerosis is described as a metabolic disease associated with a chronic low-grade inflammation linked to lipid accumulation in the intima of large and medium-sized arteries, which favors plaque formation [1,2]. Since the 1950's, mounting evidence linked cholesterol metabolism to atherosclerosis development. Atherosclerotic patients not only showed increased serum cholesterol levels, and more specifically cholesterol present in the low-density lipoproteins fraction (LDL), but also accumulation of cholesterol in macrophage foam cells pointing out to cholesterol as a culprit of immunometabolic perturbations in the establishment and development of atherosclerosis. LDL-cholesterol levels are now considered as an independent risk factor for CVDs [3,4].

Received: 29 June 2020
Accepted: 10 August 2020
Published: 17 August 2020

Interestingly, LDL accumulation into the arterial wall is associated with inflammatory signals which trigger the attraction of myeloid cells such as dendritic cells (DC), neutrophils, macrophages and monocytes [5]. Advanced atherosclerotic plaques are complex structures containing lipids, necrotic cores, calcification zones and immune cells [6]. Plaque growth increases arterial stiffness and could be responsible for disturbed blood flow, while their rupture can lead to ischaemic strokes and transient cerebral ischaemic attacks [7].

During the past two decades, tremendous progress has been made highlighting the involvement of immune cells at all stages of the disease including plaque initiation, development and rupture. Particularly, the accumulation of myeloid cells in human atheromatous plaques is a strong marker of plaque instability and predictor of negative outcome [8,9]. The respective role of the different myeloid cell types in the establishment and progression of the disease has since been thoroughly investigated using pre-clinical mouse models. Monocytes, which enter atheromatous plaques from the blood circulation and differentiate into macrophages, are the main culprits of atherosclerosis development. Further mechanistic complexity came later with the realization of the involvement of neutrophils and DCs in the disease, the latter driving and bringing adaptative immunity in the picture.

Myeloid cell precursors have diverse origins: some emerge from hematopoiesis in the bone marrow, while others arise from primitive embryonic structures [10]. Their functional diversity is thought to be acquired via the action of tissue-specific cues but little is known on how this local developmental imprinting of myeloid cells takes place [11]. This observation particularly stands regarding myeloid cell immunometabolism, as microenvironmental signals can trigger rapid metabolic adaptations to adjust the immune response. In atherosclerosis, myeloid cell metabolism influences plaque development [12]. For example, under inflammatory conditions, macrophages display increased glycolytic metabolism and Glut1 expression, the main myeloid cell glucose transporter [13]. Monocytes and macrophages from atherosclerotic patients show increased mitochondrial oxygen consumption rate (OCR). Together these findings suggest a global change in metabolic activation state [14].

A metabolomics-based analysis of human carotid plaques revealed a correlation between plaque metabolic signatures, namely elevated glycolysis and low fatty-acid oxidation, and the presence of plaque instability features [15]. This observation strongly suggests that intra-plaque metabolic cues may determine the outcome of the disease. A better understanding of how metabolites affect in situ myeloid cell activation would help for the design of new therapeutic approaches to prevent and treat atherosclerosis. In this review, we will address how metabolic signals impact on myeloid cell diversity and function in the context of atherosclerosis.

IMMUNE CELL DIVERSITY IN PLAQUES

Pioneering studies revealed that human plaques contain a variety of immune cells. These observations were repeated in mouse pre-clinical models of atherosclerosis development, namely Ldlr$^{-/-}$ and ApoE$^{-/-}$ mice. Although wild-type mice are protected against the disease, high cholesterol diet feeding of Ldlr$^{-/-}$ and ApoE$^{-/-}$ mice promotes hypercholesterolemia and atherosclerosis development. Descriptions of plaque immune cells were initially based on immunochemistry and demonstrated the presence of macrophages, B and T cells in plaque lesions [16]. Nevertheless, the limited number of parameters available was not adapted to grasp the full spectrum of immune cells residing in advanced plaques. With the improvement of flow cytometry, the number of parameters simultaneously analyzed progressively increased and a further complexity in plaque immune cell populations emerged [17,18]. Single-cell RNA-Seq and Cytometry by Time of Flight (CyTOF) technologies further extended our ability to discover and characterize new tissue-resident immune populations and their activation states. This technological leap offered a new perspective to decipher in greater depth immune cell diversity in atherosclerotic plaques [19]. In the past two years, multiple studies applied single-cell RNA-Seq techniques to human plaque samples from endarterectomy patients [20], and to aortic cells extracted from wild-type and atherosclerotic mice [18,21–25]. This generated an extensive characterization of the blood vessel-residing immune landscape in health and disease (**Tables 1 and 2**). Interestingly, only a small number of myeloid cells (of which around 70% were monocytes) were observed at steady state in the aortic wall of wild-type mice [25]. It seems reasonable that monocytes crawling on endothelial cells were the main population of immune cells detected in those studies. Leukocyte diversity was shown to greatly increase in the aorta during atherosclerosis development, as neutrophils, T cells, B cells and NK cells were also identified in Ldlr$^{-/-}$and ApoE$^{-/-}$ mice fed a chow or a high fat diet [21,22,24]. This diversity was also observed in human samples [20,22].

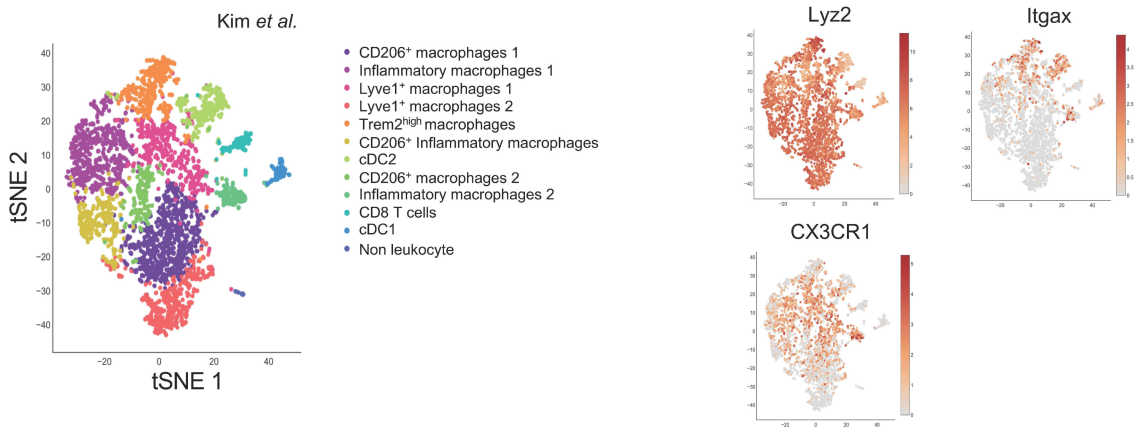
Table 1. Recent single-cell based studies assessing plaque composition.

References	Samples
Kim K et al., 2018 [23]	LdlR$^{-/-}$ mice; 12 weeks HFD; aortic CD45^{+} cells
Cochain C et al., 2018 [21]	LdlR$^{-/-}$ mice; 12 weeks HFD; aortic CD45^{+} cells
Winkels H et al., 2018 [22]	LdlR$^{-/-}$ mice; 12 weeks CD or HFD; aortic CD45^{+} cells
	Transcriptomic data; 126 samples from the biobank of Karolinska Endarterectomies
Fernandez DM et al., 2019 [20]	Endarterectomy plaque samples
Cole JE et al., 2018 [24]	ApoE$^{-/-}$ mice; CD or HFD 12 weeks; aortic CD45^{+} cells
Kalluri AS et al., 2019 [25]	WT digested aorta

Table 2. Summary of the reported plaque leukocyte proportions across the reports mentioned in Table 1.

Refs	Samples	Method	Macrophages	Monocytes	Dendritic cells			Neutrophils	T cells		B cells	NK cells
					pDC	cDC1	cDC2		$CD4^+$	$CD8^+$		
[23]	Male $LdlR^{-/-}$ HFD 12 weeks	Single cell RNA-Seq	83.9%*	ND	ND	2.2%*	6.9%*	ND	3%*		ND	ND
[21]	Male $LdlR^{-/-}$ HFD 11 weeks	Single cell RNA-Seq	28.9%	12.3%	14.9%			2%	8.7%	19.6%	2%	4%
	Male $LdlR^{-/-}$ HFD 20 weeks		49.6%	ND	14.2%			ND	8.5%	16.8%	2.1%*	2.4%*
[22]	Male $LdlR^{-/-}$ CD	Single cell RNA-Seq	13.6%	Myeloid cells: 6.2%					54.1%		24.4%	1.7%
	Male $LdlR^{-/-}$ HFD 12 weeks		27%	Myeloid cells: 21.1%					45.8%		4%	2.1%
	Female $ApoE^{-/-}$ CD		4.9%	Myeloid cells: 10.3%					60.6%		21.9%	2.4%
	Female $ApoE^{-/-}$ HFD 12 weeks		9.6%	Myeloid cells: 12.6%					49%		27.2%	1.6%
	Human	Bulk RNA-Seq deconvolution	50%*	12%	ND			ND	20%*		10%*	5%
[20]	Human	CyTOF	10.6%	2.5%	0.4%	0.1%	ND	0.1%	31.6%	31.1%	2.6%	4.1%
	Human	CITE-Seq	16%	ND	6%*	1.5%*	ND	ND	20%*	26%*	8% *	11%*
[24]	$ApoE^{-/-}$ sex unspecified CD	CyTOF	60%	2.5%	0.25%	1.6%	8.5%	2.5%	3%	3%	8%	1%
	$ApoE^{-/-}$ sex unspecified HFD 12 weeks		57%	7%	1%	1.8%	6.5%	4%	3%	3%	5%	0.75%
[25]	Female WT CD	Single cell RNA-Seq	23%*	73.4%*	3.3%*			ND	ND		ND	ND

When possible, we reported the proportion of each cell type among total leukocytes as indicated by the authors. Stars (*) indicate missing information that was estimated and completed using the Single-Cell Explorer software (Artyomov Lab, Washington University St Louis). WT = Wild-Type. CD = Chow Diet. HFD = High Fat Diet. ND = Not Determined.



A **B**

Figure 1. Single-Cell approaches highlight plaque immune cell diversity. (**A**) Single-Cell RNA-Seq of aortic CD45^{+} cells from Ldlr$^{-/-}$ mice fed a HFD for 12 weeks. Data from Kim et al. [23] (GSM3215435) were analyzed using the Single-Cell Explorer software. **List of markers used**: CD206^{+} Macrophages: Fcgr1, Itgam, Mafb, Mrc1. Inflammatory macrophages: Fcgr1, Itgam, Mafb, NLRP3, IL1b, Nfkbia. Lyve1^{+} macrophages: Fcgr1, Itgam, Mafb, Lyve1. TREM2^{high} macrophages: Fcgr1, Itgam, Mafb, TREM2, ABCG1, Lpl, Lipa. CD206^{+} Inflammatory macrophages: Fcgr1, Itgam, Mafb, Mrc1, NLRP3, IL1b (low), Nfkbia, TNF. CD8 T cells: Lck, CD3, CD8. cDC2: Zbtb46, Itgax, Flt3, Itgam (+), Itgae (−). cDC1: Zbtb46, Itgax, Flt3, Itgam (−), Itgae, IRF8. Non leukocyte: Ptprpc (−). (**B**) Expression pattern of genes used for targeted Cre expression in myeloid cells.

However, the relative proportion of each cell type reported by different single-cell studies shows significant fluctuations. In mice, macrophages were reported to represent from 9 to 80% of the aortic leukocyte pool and, inversely, T cells represented from 3 to 60% of leukocytes. The same variations were observed in human samples (Table 2). These differences could be explained by multiple factors, and notably differences in tissue digestion technique, leukocyte purification method, and the markers and transcripts used for cell type identification. The entry of atherosclerosis research in the single cell RNA-Seq era could therefore benefit from a universal experimental pipeline that would facilitate comparison between studies. A first step toward this direction could consist in generating a meta-analysis of the data from the studies summarized in Table 1 to characterize plaque composition. A meta-analysis study was recently published and evidenced the immune cell diversity in plaque and the markers allowing to define each population [26].

These studies illustrate that single-cell approaches are amongst the most powerful tools to precisely identify cell subsets as well as their respective metabolic demands. Myeloid cells were broadly shown to represent a significant part (up to 90%) of immune cells in atherosclerotic lesions [23]. Here, we will briefly discuss the identity and origin of plaque resident myeloid cells.

Monocytes

Monocytes are short-lived mononuclear phagocytes that are generated in the bone marrow (BM) during hematopoiesis [27,28]. They rely on CSF1R (Colony Stimulating Factor 1 Receptor) signaling for their development and survival [29,30]. Among leukocytes, which have been positively correlated with cardiovascular events in humans [31–33], monocytes play a pivotal role in atherosclerosis development. Hypercholesterolemia, a key component of atherosclerosis, has been associated with increased circulating monocyte numbers (monocytosis) in mice, rabbits, swines and humans [34–36].

Circulating monocytes are identified as $CD11b^{+}$ $CD115^{+}$ cells, and exist as two functionally distinct subsets in both mice and humans: classical monocytes, identified as $Ly6C^{high}$ in mice and $CD14^{high}$ $CD16^{low}$ in humans, and non-classical monocytes, identified as $Ly6C^{low}$ and $CD14^{low}$ $CD16^{high}$ [37]. An additional human population of $CD14^{+}CD16^{+}$ monocytes has also been documented. Developmentally, $Ly6C^{low}$ monocytes were proposed to arise from $Ly6C^{high}$ monocytes [38,39]. $Ly6C^{low}$ monocytes require the transcription factor Nr4a1 (Nur77) for their maturation [40], they are commonly referred to as “patrolling monocytes”, as they are closely associated to the endothelium which they survey in order to remove dead endothelial cells [41,42].

$Ly6C^{high}$ monocytes are also called “inflammatory” monocytes, as they accumulate during infections and are preferentially recruited to inflamed tissues [38]. They display a high expression of CCR2 [43,44], the main chemokine receptor governing monocyte recruitment to inflammatory sites [45] as well as into atherosclerotic plaques. Plaque initiation is driven by $Ly6C^{high}$ monocyte recruitment to the intima of the endothelial wall [46–50]. Their inflammatory nature seems to be supported by the fact that osteopetrotic mice, which lack functional CSF1 (Colony Stimulating Factor 1) and therefore monocytes [51], are protected against hypercholesterolemia-induced atherosclerosis [52–54]. In 1998, two independent studies documented the key role played by monocyte recruitment to atheromatous lesions through the CCL2-CCR2 chemotactic axis, as mice deficient for CCR2 [55] or its ligand CCL2 [56] also displayed reduced atherosclerotic lesions. Monocyte chemotaxis in atherosclerosis was further characterized in 2007, as Tacke and colleagues provided evidence of the relative contribution of CCR2, CCR5 and CX3CR1 for monocyte recruitment into plaques [47]. CX3CR1 is highly expressed on $Ly6C^{low}$ monocytes and remains detectable on $Ly6C^{high}$ monocyte subset [57]. In contrast, CCR2 is predominantly expressed by the $Ly6C^{high}$ population [57].

Understanding the roles played by metabolism in monocyte biology is of key importance considering the limitations of current therapies. Indeed, limiting monocyte recruitment to the plaque seems to be a reasonable strategy for reducing the development of atherosclerotic lesions. Complementarily, new anti-inflammatory approaches have recently

gained great interest. Indeed, lowering the inflammatory response has emerged as a novel therapeutic target to decrease CVDs related death and comorbidities. Interleukine-6 (IL-6) and IL-1β are two well-established pro-inflammatory mediators and their levels are increased during atherosclerosis progression [58]. Among those two, the pro-inflammatory cytokine IL-1β emerged as a major mediator of atherosclerosis development [59–61]. Surprisingly, IL-1β signaling was also shown to be an important component of plaque remodeling and stability [62]. The Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) trial showed that patients treated with the IL-1β-targeting monoclonal antibody Canakinumab had a lowered cardiovascular-event-related mortality rate. However, Canakinumab treatment induced various side effects and increased susceptibility to infections, resulting in no overall survival benefits and underlining the urgent need for other therapeutic approaches [63,64].

Macrophages

Macrophages are highly phagocytic cells that can be identified through their co-expression of CD64 and MerTK [11]. Macrophages are ubiquitously present across organs and play key roles both in health and disease [11]. A key function of tissue-resident macrophage is the removal of dead cells, a process named efferocytosis [65]. Every day around 0.4% of the total estimated number of 3.7×10^{13} cells dye in the adult human body [66]. Although all macrophage populations perform efferocytosis, they also display tissue-specific functions such as heme detoxification and iron handling in the spleen, surfactant clearing in the lungs or thermogenesis regulation in brown adipose tissue [67,68]. This functional heterogeneity can partly be explained by the developmental origin of macrophages. Microglia, the population of brain resident macrophages, arise from yolk sac precursors present at early developmental stages [69,70]. Alveolar pulmonary macrophages originate from foetal liver progenitors [71] while the population of gut macrophages derives from bone marrow precursors [72]. Consequently, embryonically-derived macrophages and monocyte-derived macrophages often coexist in adult tissues [73,74]. Like monocytes, tissue-resident macrophages rely on CSF1R signalling, which can bind either IL-34 or CSF1, for their maintenance. Interestingly, a tissue specificity for either ligand has been observed among tissue-resident macrophages, as microglia rely on IL-34 while large peritoneal macrophages rely exclusively on CSF1 [75,76]. Furthermore, macrophage heterogeneity can be attributed to local environmental features, even between subsets that share a common developmental origin [11].

Atheromatous plaque macrophages are monocyte-derived macrophages with the ability to proliferate in situ following their recruitment and differentiation. The understanding of macrophage diversity in metabolic disorders and inflammatory diseases is of particular

interest during atherosclerosis, as multiple macrophage subsets with specific immune profiles and functions have been observed within the plaque [18,21–23] (Figure 1A). Our knowledge on plaque macrophage diversity was previously restricted to pro-inflammatory "M1" macrophages, anti-inflammatory "M2" macrophages, and foam cells which were considered inflammatory cells [5]. The in situ identification of these subsets was based on immunohistochemistry approaches, while their functions were explored using in vitro models. However, new single cell methods have now revealed more layers of complexity in plaque macrophage subsets [18,21–23]. Notably, the single-cell RNA-Seq dataset from Kim and colleagues [23], which displays the greater myeloid cell enrichment, shows the existence of several distinct populations of inflammatory and anti-inflammatory subsets. Surprisingly, expression of the archetypical anti-inflammatory macrophage marker CD206 also appears on populations expressing inflammatory markers (Figure 1A). Two macrophage populations expressed high levels of Lyve1, a marker associated with tissue-resident macrophages [77] which were also identified by Cochain et al. [21] (Figure 1A). This new technology also allowed a detailed in vivo characterization of foam cells, identified as Trem2^{high} macrophages [23,78,79].

Single cell studies now pave the way to understanding plaque macrophage biology. Further investigation is needed to determine how these different subsets participate to inflammation or its resolution via efferocytosis and plaque remodelling [19]. To establish the developmental connection between plaque macrophage populations and their particular localization and metabolic demands is of crucial significance to understand the pathological mechanisms occurring during atherosclerosis progression.

Dendritic Cells

DCs are professional antigen presenting cells (APC). Two major DCs populations have been identified in mice and humans: the conventional (cDCs) and the plasmacytoid dendritic cells (pDCs). Both human and mouse cDCs highly and selectively express the transcription factor Zbtb46 (Zinc finger and BTB domain containing 46) [80,81]. Zbtb46 is not expressed by other myeloid cells such as macrophages, monocytes or neutrophils. In mice, cDCs populations highly express CD11c and MHC II and two main subsets have been identified in lymphoid and non-lymphoid tissues. In lymphoid tissues, cDC1s express CD8, CD24 and XCR1 while cDC2s are characterized by CD4 and Sirpα expression [82]. In the majority of nonlymphoid tissues, cDC1s are described as CD103$^+$ XCR1$^+$ and cDC2s as CD11b$^+$ Sirpα$^+$. cDC1 and cDC2 require specific transcription factors for their development. cDC1 depend on BATF3 (Basic Leucine Zipper activating transcription factor–like transcription factor 3) and IRF8 (IFN regulatory factor 8) while cDC2 rely on IRF4 and Notch2 for their development and maintenance [82]. A key feature of cDCs is their high

capacity to capture antigens in peripheral tissues and subsequently migrate to local draining lymph nodes to initiate the adaptive immune response. Another major function of cDCs is the production of pro-inflammatory cytokine such as IL-6, TNFα and IL-1 following activation of innate immunity receptors. This cytokine production leads to immune cell recruitment and mobilization and allows for specific and efficient immune responses. On the other hand, pDCs essentially release type 1 interferons (IFN-I), both IFNα and IFNβ, in response to virus infections [83]. Their potential implication in atherosclerosis is suggested by the fact that IFN-I decreases macrophage phagocytic abilities [84] and that IFNAR-deficient animals have decreased plaque area and macrophage content [85].

In the context of atherosclerosis, cDCs contribute to chronic inflammation by attracting and activating T cells [86]. The production of CCL17 by mature cDCs contributes to $CD4^+$ T cells and regulatory T cells (Tregs) migration and recruitment to plaques. CCL17 deletion leads to a slower atherosclerosis progression and a decreased number of macrophages and T cells in plaques [87]. The presence of $CD4^+$ T cells with a phenotype of antigen activated ($CD44^+$) cells was documented in mouse atherosclerotic models [17]. $CD4^+$ T cells stimulation requires a peptide loading on major histocompatibility complex (MHC II), selectively expressed by antigen presenting cells. The cDC antigen presentation function seems to play a pivotal role in the progression of atherosclerosis. Nevertheless, and despite recent progress in the field, the nature of the antigen (peptide or lipid) remains to be fully understood. For instance, ApoB (the core protein in LDL) reactive $CD4^+$ T cells were identified in pre-clinical atherosclerotic models [17] and humans [88]. Immunization strategies were developed using ApoB epitopes and those demonstrated atheroprotective effect, illustrated by reduced plaque area, when conjugated to appropriate adjuvants [89,90]. This protection was associated with increased IL-10 production, an anti-inflammatory cytokine mainly secreted by regulatory T cells (Tregs). In atherosclerotic patients, an oligoclonal T cell repertoire was observed in comparison to healthy patients [91,92]. This observation further supports the relevance of antigen presentation during disease development. Recently, the generation of MHC II tetramers loaded with ApoB-derived peptide revealed that the majority of ApoB-recognizing T cells are T regs [88]. Moreover, the deletion of two important costimulatory molecules: CD80 and CD86 in mice DCs decreased T-cell activation/infiltration in plaques [93] demonstrating that cDCs play a crucial role during disease development.

In advanced plaques, apoptotic cell accumulation due to defective efferocytosis leads to DNGR-1 activation (dendritic cell NK lectin group receptor-1) on $CD8a^+$ cDC1s, which blunts IL-10 production, therefore contributing to atherosclerosis aggravation [94]. However, the mechanisms underlying the defective efferocytosis in DCs are still unknown and need to be deciphered. In conclusion, DCs, as pivotal players

linking innate and adaptive immunity, offer new insights that may lead to new therapeutic targets and notably vaccination strategies.

Neutrophils

Neutrophils are associated with the early inflammatory response [95]. Neutrophils have been shown to either be able to directly affect atherogenesis [96], or contribute to pathology onset by driving immune cell entry in atherosclerotic lesions [97] and by promoting plaque rupture [98] respectively. This suggests an important crosstalk between neutrophils and other immune and stromal cells.

Growing evidence suggests that neutrophils play a pivotal role in the initiation of atherosclerosis. Neutrophil adhesion to the endothelial wall through CCL3 and CCL5 binding on CCR1, CCR3 or CCR5 triggers neutrophils extravasation and their entry into plaques [99]. There, activated neutrophils release granule proteins containing chemotactic “alarmins”, such as cathelicidin/LL-37 in Human (CRAMP in mice), Human α-defensins (human neutrophil peptides, HNPs), azurocidin (HBP, CAP37) and serprocidins (elastase, cathepsin G, proteinase-3), inducing leukocytes attraction and recruitment to the site of inflammation (for review see [100]). In addition, S100A8/A9, a cytoplasmic protein, reduces neutrophils rolling on the endothelial wall and activates β2 integrin to facilitate leukocyte extravasation and entry to the site of inflammation [101]. Interestingly, alarmins have also been reported to contribute to the activation of inflammasomes such as NLRP3 [102]. NLRP3 activation leads to IL-1β and IL-18 production and to the HMGB1 alarmin (High-mobility group box 1 protein) release, creating a loop that amplifies innate immune responses [103]. NLRP3 inflammasome activation then increases neutrophil recruitment to inflammatory sites leading to the activation of neutrophil extracellular traps (NETs) [104]. NETs are web-like fiber structures released by neutrophils and made of extracellular chromatin, nuclear proteins, and serine proteases. NETs are known to increase monocyte recruitment to inflamed sites and trigger reactive oxygen species (ROS) and proinflammatory cytokines release by macrophages [105,106]. In this context, NETs may promote type I interferon (IFN-I) release from pDCs contributing therefore to atherosclerosis progression and suggesting an essential crosstalk between neutrophils and pDCs [107].

Neutrophils have been found at sites of plaque rupture in patients with acute coronary syndrome [108]. Interestingly, neutrophils are essentially located in the unstable layers of human atherosclerotic lesions with a high inflammatory activity and also correlated to the elevated numbers of monocytes found in these regions [99]. In addition, NETs are thought to be involved in plaque destabilization through the induction of endothelial cell wall cytotoxicity in humans [109,110]. Neutrophils are the main producer of myeloperoxidase (MPO) [111]. MPO is a heme-containing peroxidase that catalyzes the formation of reactive oxygen species intermediates [112] that induce macrophage cholesterylester

accumulation and foam cell formation, leading to atherosclerosis aggravation [113]. Recent studies have highlighted that neutrophils undergo transcriptional regulations under inflammatory conditions and NETosis [114,115]. The significance of NETs during atherosclerosis was extensively described in a recent manuscript [115].

Mouse Models

Mouse Cre-Lox systems have extensively been used to explore the role of myeloid cell functions in atherosclerosis. $Lyz2^{Cre}$, $CX3CR1^{Cre}$ and $CD11c^{Cre}$ mice were the most commonly used to study macrophages and neutrophils, monocytes, and dendritic cells respectively. Although these genes are dominantly expressed by the aforementioned cell types, some well-documented overlaps in their expression exist between myeloid cell types. Single-cell sequencing approaches have now brought to light the subset-specific expression pattern of these genes within the plaque (Figure 1B), which may allow more specific targeting of myeloid subsets within plaques and reinterpretation of previously generated data.

As expected, $Lyz2^{Cre}$ appears to be virtually ubiquitously expressed across plaque resident myeloid cells. Although CX3CR1 expression is only restricted to certain myeloid subsets within the plaque, most macrophages are monocyte-derived cells which therefore expressed CX3CR1-driven Cre at an earlier differentiation stage. However, the use of inducible $CX3CR1^{CreERT2}$ models gives more flexibility to the model. As an example, Lin and colleagues recently used $Cx3cr1^{CreERT2\text{-}IRES\text{-}YFP/+}Rosa26^{fl\text{-}tdTomato/+}$ mice in a fate-mapping and single-cell approach [18]. The authors induced Cre expression when plaques were established, immediately prior to plaque regression induction in order to differentially characterize $CX3CR1^{+}$ plaque cells and cells derived from $CX3CR1^{+}$ precursors [18]. $CD11c^{Cre}$ mice were extensively used to characterize DCs functions in health and disease. However, CD11c was also shown to be expressed by $Ly6C^{low}$ monocytes [57] which can, to a lower extent than $Ly6C^{high}$ monocytes, infiltrate atheromatous lesions [47]. As shown in Figure 1B, plaque expression of CD11c is not restricted to DCs, but also concerns certain macrophage subsets including the now well identified $TREM2^{high}$ foam cells [23,78,79]. $CD11c^{Cre}$ mice could therefore be a valuable model to study foam cell metabolism during atherosclerosis.

METABOLIC PHENOTYPE OF PLAQUE IMMUNE CELLS

Atherosclerosis progression is accompanied by a modulation of systemic and plaque metabolites. Recently, non-invasive imaging techniques, commonly used in oncology, were deployed to predict rupture-prone plaques. Positron emission tomography (PET) is traditionally employed to investigate myocardial reperfusion. PET/CT studies revealed an accumulation of the glucose analog 18F-fluoro-2-deoxy-d-glucose (18F-FDG) in atherosclerotic lesions in humans [116]. This suggested increased glucose avidity and potentially metabolization in

plaque residing cells. A metabolomic analysis performed on iliac-femoral arteries extracted from control and atherogenic rabbits revealed an increased abundance of glycolysis and pentose phosphate pathway (PPP) metabolites in plaque-enriched vessels [117]. Whether these metabolites accumulate in specific immune or stromal cell-type remains to be established. In the following section we will discuss the impact of myeloid cell glucose metabolism on plaque development.

Lipid Handling

Monocytes

Hypercholesterolemia, the predominant metabolic feature of cardiovascular diseases, is known to influence hematopoiesis and induce a differentiation bias of hematopoietic stem cells (HSCs) towards the myeloid lineage. Indeed, HSCs obtained from ABCA1$^{-/-}$ ABCG1$^{-/-}$ mice, lacking transporters involved in cholesterol efflux, display increased proliferation and myelopoiesis switch [118]. This phenomenon is amplified in ApoE$^{-/-}$ mice, one of the most commonly used murine models of hypercholesterolemia-induced atherosclerosis [119]. Taken together, recent data on cholesterol-related myelopoiesis exacerbation suggest that an increase in cellular cholesterol content promotes membrane lipid raft formation in HSCs, thus promoting the stabilization of chemokine and cytokine receptors at the cell surface and signal HSCs to quit quiescence [118,120–122].

Emerging immunometabolism-centered studies have mainly focused on macrophages, while only a few studies investigated monocyte metabolic requirements. This may partly be explained by technical difficulty of using undifferentiated monocytes in *in vitro* cultures. Recent studies pointed towards lipid metabolism as an important factor of monocyte homeostasis. Using a BM transplant approach, Babaev and colleagues reported a decreased CCR2 expression on blood monocytes from Ldlr$^{-/-}$ mice with FABP5$^{-/-}$ (Fatty-acid binding protein) hematopoietic compartment, suggesting a chemotaxis-dependent proatherogenic role of myeloid FABP5 expression [123]. FABPs regulate intracellular lipid traffic and control their access to specific organelles. By contrast, FABP4 deletion in immune cells had no impact on plaque development [123]. ApoE$^{-/-}$ mice with myeloid-specific deletion of lipoprotein lipase (Lpl) displayed decreased plaque development [124]. Lpl hydrolyzes circulating TGs and control their levels. Impaired monocyte generation and differentiation to macrophages were observed in a mouse model of Lpl deficiency and were attributed to Lpl-dependent regulation of CSF1R signaling [125]. Interestingly, FABP5 and Lpl mRNA were highly and selectively expressed in Trem2^{+} foam cells (Figure 2A,B). This could suggest that the atheroprotective effects of myeloid-specific deletion of Lpl might not be caused solely by monocytes but could additionally be the consequence of foam cell dysfunction.

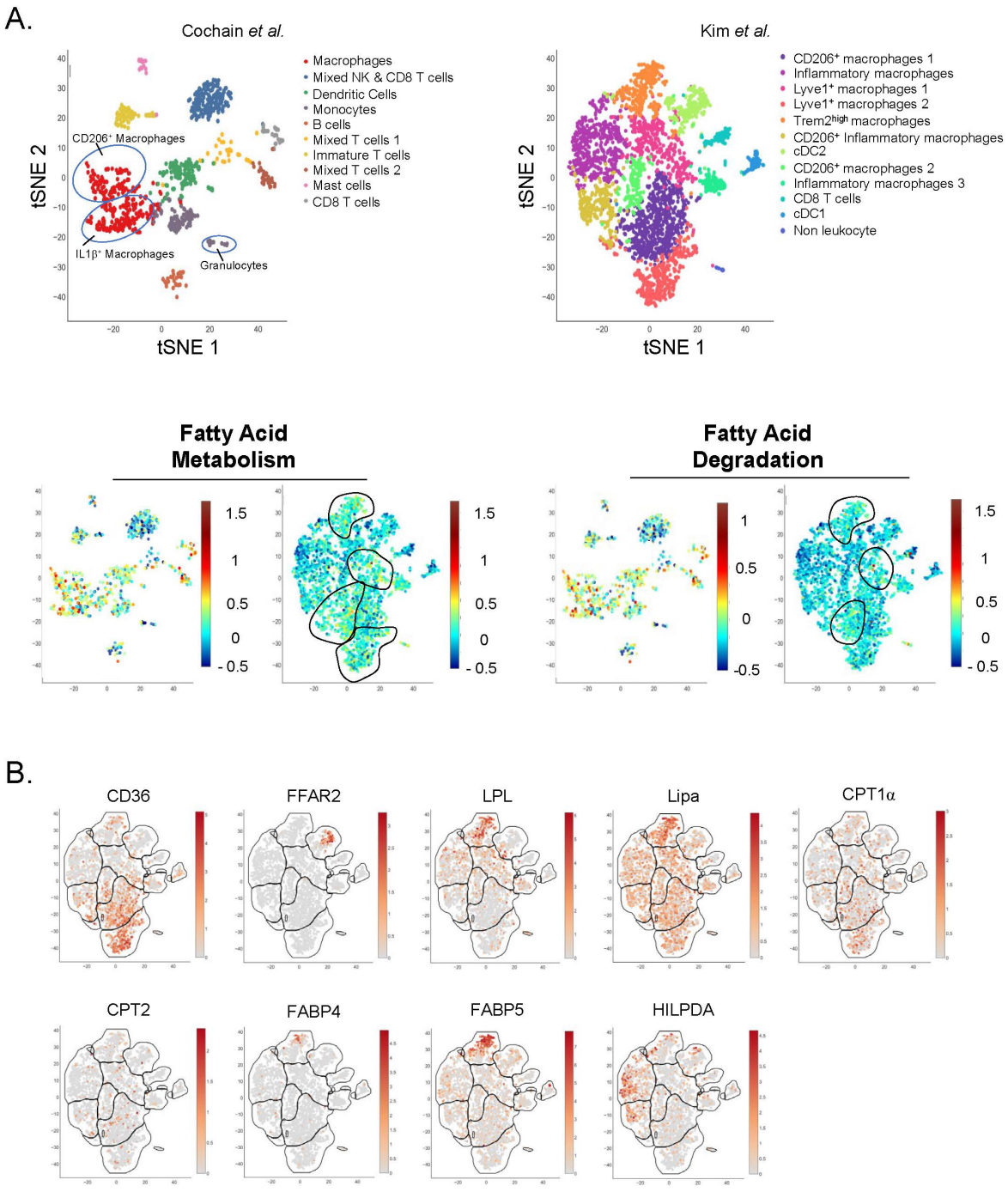


Figure 2. Single-Cell analysis of plaque immune cell lipid metabolism. **(A,B)** Single-Cell RNA-Seq of aortic CD45⁺ cells from Ldlr$^{-/-}$ mice fed a HFD for (left) 11 weeks or (right) 12 weeks. Data from (left) Cochain et al. [21] (GSE97310) and (right) Kim et al. [23] (GSM3215435) were analyzed using the Single-Cell Explorer software (Artyomov lab). **(A)** Leukocyte clusters and corresponding KEGG Metabolic Pathway analysis. Fatty-Acid metabolism: KEGG mmu01212. Fatty-Acid degradation: KEGG mmu00071. **List of markers used in Cochain et al.**: Macrophages (mixed subsets): Itgam, Fcgr1, MerTK. Mixed NK and CD8 T cells: CD3, CD8, Klrb1c, Ncr1, Gzmb. Dendritic Cells (mixed subsets): Itgax, Ciita, Zbtb46. Monocytes: Itgam, Fcgr1, Ly6C, CCR2. B cells: Ciita, CD19, CD79α/β. Mixed T cells: Lck, CD3, CD4, CD8, Rag1. Immature T cells: Lck (−), CD3(+), Rag1 (−), CD4 (−), CD8 (−). Mast cells: Furin, Il1rl1. CD8 T cells: Lck, CD3, CD8. **Markers used in Kim et al. are in the legend for Figure 1A.** **(B)** Expression pattern of genes involved in lipid metabolism (Kim et al. [23] (GSM3215435)).

The importance of monocyte lipid metabolism was challenged by recent data. Jordan and colleagues observed a decrease in blood monocyte numbers during fasting in humans. This effect could not be reverted by fat supplementation in mice, while carbohydrate and protein supplementation both restored blood monocyte counts [126]. However, this modulation of monocyte counts was attributed to modulations of CCL2 production through a liver-BM axis, and not to monocyte cell-intrinsic mechanisms. Nevertheless, monocytes from fasted mice were in a pronounced quiescent metabolic state in comparison to controls, as extracellular flux analysis of these cells showed reduction in both oxygen consumption rate and extracellular acidification rate. This was associated with up-regulation of inositol triphosphate metabolism and suppression of serine and glutathione metabolism. Overall, this metabolic adaptation of monocytes to fasting was associated with improved outcomes in models of chronic inflammatory diseases [126].

As atherosclerosis is associated with numerous systemic metabolic alterations, these exciting results support the urgent need to identify the dietary-related metabolic mechanisms controlling monocyte inflammatory and migratory potentials in this disease. Indeed, both qualitative and quantitative diet modulations could then be envisioned as non-invasive prophylactic therapies for patients presenting monocytosis and metabolic syndrome.

Macrophages

Lipid-laden macrophages were first described inside of atherosclerotic lesions in the late 1970s [127]. These macrophages, called foam cells, take up excessive cholesterol and oxidized LDL (oxLDL) particles via their scavenger receptors, which leads to the intracellular formation of lipid droplets [128–131]. The presence of these cells is considered as a hallmark of atherosclerosis, and foamy macrophages have historically been held culprit for plaque progression.

Intracellular accumulation of cholesterol has been linked to foam cell formation, cytokine production and atherosclerosis progression in a mouse model of defective cholesterol efflux [132]. Atherosclerosis progression has been associated with the formation of cholesterol crystals, which results from reduced esterification of free cholesterol [133]. These crystals can trigger inflammation through the activation of the NLRP3 inflammasome and subsequent IL-1β maturation, which have lately been a major focus of atherosclerosis research [134]. These results support a beneficial role for cholesterol efflux, which is mediated through the liver X receptor (LXR)-regulated transcriptional control of among others the cholesterol transporters ABCA1 and ABCG1. Mice deficient for LXRα and LXRβ display the formation of atheromatous lesions containing foam cells even in the absence of diet-induced hypercholesterolemia [135]. Subsequently, attempts have been made to decrease intracellular cholesterol accumulation and foam cell formation by the use of synthetic

LXR agonists which have proven to be beneficial in pre-clinical models of atherosclerosis [136–138]. Consistently, myeloid-specific deletion of the LXR-regulated ABCA1/G1 cholesterol transporters was shown to exacerbate atherosclerosis development [139]. Alternatively activated (M2) human monocytes and macrophages were reported to be less responsive to LXR agonists [140]. Interestingly, they also displayed less foam cell traits despite decreased ABCA1 expression. This was countered by an improved cholesterol esterification capacity (an anti-inflammatory mechanism) [141], suggesting that efficient cholesterol handling may be more valuable than cholesterol efflux [140]. PPARα stimulation positively regulates ABCA1 expression [142]. Additionally, PPARγ activation also increases ABCA1 expression via LXR [143]. PPARα prevents foam cell formation and decreases plaque development [144,145]. PPARγ agonist also decreased foam cell formation [144]. The role of PPARs in macrophage biology and atherosclerosis is extensively reviewed in [146,147]. However, whether and how precisely PPARs affect specifically different myeloid cell populations in plaque remains to be defined. We recently reported that lysosomal acid lipase (LIPA)-dependent cholesterol hydrolysis promotes macrophage efferocytic capacity [148]. LIPA is expressed by all plaque macrophage subsets and is particularly enriched in foam cells (Figure 2B). The relevance of our observations linking LIPA activity and efferocytosis to atherosclerosis needs to be further investigated, as multiple studies reported a correlation between LIPA variants and coronary artery disease [149,150].

Macrophage foam cell formation is regulated by natural antibodies recognizing modified LDL particles and apolipoproteins. Indeed, the inhibition of oxLDL uptake by oxLDL-specific natural IgMs, which mask oxidized epitopes, decreases foam cell formation, inflammation and atherosclerosis development [151–153]. The production of oxLDL-specific antibodies, both of the IgM and IgG isotype, occurs during the development of atherosclerosis [154]. IgG-containing immune complexes are recognized by FcγR receptors, and $ApoE^{-/-}$ mice deficient for FcγRIIb/CD32b, a low affinity inhibitory receptor, showed reduced plaque lipid content suggesting lower foam cell formation [155]. This was associated with an overall increase in plaque stability, as well as in circulating levels of oxLDL and IgG-ApoB immune complexes, suggesting a lower uptake of these particles by macrophages [155]. Foam cell formation was also reported to be induced by ApoA1-specific IgG in vitro [156]. Although atherosclerosis-associated IgMs and IgGs display different roles in foam cell formation, only the former are robustly associated with a (protective) role in the development of the disease [157].

In macrophages, a consequence of foam cell formation could be lipid overload-induced toxicity, which may hypothetically be a determinant of plaque necrosis. Both fatty acid metabolism and degradation pathways are enriched in plaque macrophages (Figure 2A). The hypoxia-inducible lipid-droplet-associated (Hilpda) protein has emerged as a key player in lipid

droplet handling. Its role as a lipid-sensor and inhibitor of ATGL (the rate limiting enzyme of adipose tissue lipolysis) promotes lipid accumulation into lipid droplets and foam cell formation [158]. In plaques, Hilpda mRNA was preferentially expressed in inflammatory macrophages (Figure 2B). This Hilpda-mediated lipid storage was reported to be necessary for the maintenance of macrophage viability upon lipid overload, suggesting a beneficial role for lipid storage in terms of survival [159]. Nevertheless, Hilpda deficiency was shown to decrease atherosclerosis development and plaque lipid content, without affecting plaque macrophage apoptosis [159]. The authors attributed this phenotype to the Hilpda-dependent macrophage lipid accumulation and production of prostaglandin E2, which promotes vascular inflammation [160].

Overall, previous reports point towards a proatherogenic role for foam cells. However, the emergence of omics approaches led recent studies to challenge this view. Foamy peritoneal macrophages extracted from $Ldlr^{-/-}$ mice fed a high cholesterol diet surprisingly showed a LXR-mediated down-regulation of genes associated with inflammatory responses and chemotaxis [161]. A turning point in foam cell research was reached by Kim and colleagues, who developed a strategy to isolate and characterize plaque foam cells using a lipid probe-based strategy [23]. Surprisingly, their results showed that foam cells only represent around 10% of aortic macrophages in atherosclerotic mice, although this proportion may vary depending on isolation efficiency. Moreover, both bulk and single cell RNA-Seq approaches showed that foamy macrophages are rather anti-inflammatory in comparison to non-foamy plaque macrophages. As discussed earlier, this is of particular interest in the context of the recent CANTOS trial, as NLRP3 and IL-1β expression were clearly a feature of non-foamy macrophages [23]. While previous studies relied greatly on in vitro models to analyze macrophage lipid metabolism and foam cell formation, this innovative approach may have supplied the methodology to further characterize foam cells in vivo. As discussed earlier, studying macrophages in the context of their micro-environment has repeatedly proven to be the key to understanding their biology.

In vitro studies revealed that IL-4 induces a specific metabolic profile in macrophages. These cells are named alternatively activated (M2) macrophages and rely on fatty acid oxidation for their metabolic needs [162]. Seminal studies demonstrated that fatty acid oxidation inhibition in macrophages prevents their M2 phenotype. This concept was recently challenged by the demonstration that etomoxir, a specific Cpt1a inhibitor, has “off-target” effects even at fairly low concentrations [163,164]. Genetic Cpt2-deletion failed to affect macrophage alternative polarization, further challenging the previously established dogma [165]. Cpt1 and Cpt2 mRNA were detected in plaque-resident myeloid cells without a subset-specific signature (Figure 2B). Whether plaque resident macrophages rely on fatty oxidation and Cpt1 remains currently unknown. Importantly, macrophage alternative activation depends on CD36, a membrane receptor for long

chain fatty acids [162]. Single-cell RNA-Seq analysis revealed that CD36 is highly expressed in Lyve1^{+} plaque resident macrophages (Figure 2B). Of interest, Lyve1 is a canonical M2 activation marker which, together with CD36 expression, might help identifying the real in vivo alternatively activated macrophage relying on fatty acid oxidation (at least in the context of the plaque). CD36 is involved in non-classical monocyte patrolling during atherosclerosis induction [166]. Previous reports demonstrated that CD36 plays a crucial role during atherosclerosis development but it remains unclear how CD36 governs plaque myeloid cell metabolism [167–170]. For instance, CD36 signaling is involved in ROS generation and controls macrophage cytoskeleton organization [171].

Dendritic cells

One of the major proofs of DCs implication in atherosclerosis development is their impact on cholesterol homeostasis. Indeed, DC depletion in hyperlipidemic CD11c-DTR ApoE$^{-/-}$ mice leads to increased hypercholesterolemia but no change in atherosclerosis due to lower DC-driven T-cell activation, suggesting that there is a close relationship between DCs and cholesterol homeostasis. CD11c expression in plaque is not restricted to DCs, and this function might be shared with CD11c-expressing macrophages (Figure 1B). Increasing DC survival by overexpressing Bcl2 leads to decreased cholesterol plasma levels [172]. However, long term DC depletion led to a progressive myeloproliferative syndrome, highlighting an indirect impact on the hematopoietic system [173]. Additionally, in Ldlr-deficient mice, DCs in atherosclerotic lesions have been shown to capture oxLDL contributing to foam cell formation and therefore to atherosclerosis progression [174].

Despite the fact that pDCs are present in a relatively low frequency in human and mouse atherosclerotic plaques, this cell type also plays a role in atherosclerosis development. pDCs numbers are increased in aortas of ApoE$^{-/-}$ and Ldlr$^{-/-}$ mice fed a high-fat diet [24,175]. Intriguingly, ApoE$^{-/-}$ mice depleted in pDCs display decreased lipid-containing area, lower T cell activation and lower macrophage accumulation in the plaque [176]. In addition, when treated with oxLDL, pDCs show increased phagocytic capacity as well as a stimulated antigen-specific T cell response [177]. Genetic pDC depletion, following diptheria toxin administraton in BDCA2-DTR atherogenic mice, led to increased lesion area [175]. Moreover, TLR-induced IFN-I production by pDCs is triggered by neutrophils NETs in human atherosclerotic plaque [178]. All together, these data suggest that pDCs might be interesting targets in controlling the evolution of atherosclerosis. However, pDCs role in atherosclerosis is still under debate due to the opposite effects the antibody used against pDC bone marrow stromal cell antigen-2/PDCA1 has on Ldlr$^{-/-}$ and ApoE$^{-/-}$ mouse [176,177,179].

Neutrophils

Cholesterol metabolism appears to play a key role in neutrophil biology, as both $Ldlr^{-/-}$ and $ApoE^{-/-}$ mice fed a high fat diet display increased blood neutrophil numbers [119]. Cholesterol efflux receptors such as ABCA1/ABCG1 notably regulate neutrophil adhesion and activation [180]. Moreover, neutrophil accumulation and NETosis have also been found in the context of defective cholesterol efflux induced by ABCA1 and ABCG1 deficiency [181] (for review see [182]). Additionally, inhibition of cholesterol efflux in myeloid progenitors led to increased neutrophil production while a disruption in the chemotactic axis CXCL12-CXCR4 in the BM led to neutrophilia and therefore amplified lesion formation [97]. Moreover, mice fed a high-fat diet show significant increase in circulating neutrophil numbers [183]. However, surprisingly, epidemiological studies in humans have shown a positive correlation between elevated numbers of circulating neutrophils and cardiovascular events, independently from serum cholesterol levels [184]. In addition, fatty acids have also been proposed to be involved in neutrophils metabolic demands. Indeed, fatty acid receptors including free fatty acid receptor-1 (FFAR1/GPR40), free fatty acid receptor 2 (FFAR2/GPR43), and GPR84 are expressed on neutrophils [185]. However, short term fasting in humans had no effect on circulating neutrophil levels [126]. Cell-autonomous effects of lipids on neutrophils and their relevance in atherosclerosis require further investigations.

Glucose Metabolism in Myeloid Cells

Monocytes

Unlike tissue-resident immune cells, monocytes need to quickly adapt to their new environment after blood vessel extravasation and entry into peripheral tissues. This seems critical in atherogenic conditions, as recent evidence suggests that monocytes might contribute to the onset of the disease due to their sensitivity to the plaque micro-environment, rather than to a preexisting inflammatory phenotype. Notably, Williams and colleagues showed that newly-recruited monocytes lose motility as they differentiate into macrophages within the plaque, thus reducing their capacity to reach apoptotic cells located deeper within the plaque and perform efferocytosis [186]. This rapid adaptation probably requires an adjustment of metabolic pathways to the locally available substrates.

In humans, glucose metabolism disorders such as *diabetes mellitus* have been associated with cardiovascular diseases, though the underlying cellular mechanisms remain unclear [187,188]. The use of the glucose analog 18F-FDG in PET-CT imaging has brought to light a correlation between acute coronary syndrome and 18F-FDG accumulation (representative of glucose avidity) in the bone marrow and the spleen (the later probably reflecting extramedullary hematopoiesis) in at least two independent cohorts [189]. Interestingly, Oburoglu and colleagues showed

that in vitro (human $CD34^+$ cells) and in vivo (newborn mice), administration of 2-deoxyglucose, a partially non metabolizable glucose analog used to inhibit glycolysis, restricted myeloid differentiation while promoting erythroid differentiation of HSCs [190]. Consistently, using chimeric pre-clinical models of atherosclerosis, our group previously reported a decrease in myelopoiesis and plaque development in mice with partial deficiency for Glut1, the main glucose transporter in the hematopoietic compartment [191]. Increased glucose levels in diabetic mice drive myelopoiesis, further supporting the evidence that glucose metabolism favors myeloid cells generation [192]. Interestingly, Jordan and colleagues reported a direct relation between food intake and systemic CCL2 levels, which allows for monocyte egress from the bone marrow compartment to the blood circulation [126]. This effect was mainly attributed to glucose metabolism, as the authors observed a positive correlation between blood monocyte counts and the quantity of gavage-administered glucose. Furthermore, monocyte mobilization could be inhibited by gavage with 2-deoxyglucose [126]. As discussed earlier, the CCL2-CCR2 chemotactic axis governs monocyte recruitment to atherosclerotic plaques and progression of the diseases [47]. Importantly, monocyte CCR2 expression strongly associates with vascular wall inflammation in patients with CVD risk [193]. However, whether glucose affects chemokine receptor expression on monocytes and facilitates their entry into inflamed plaques remains to be explored. In a pre-clinical plaque regression model, it was demonstrated that lowering plasma glucose concentration prevents monocyte entry into the inflamed plaque and improves pathology resolution [192]. Nevertheless, whether glucose lowering therapies affect monocyte CCR2 expression and their ability to enter into plaques and differentiate into macrophages also requires further investigations.

Macrophages

Macrophages rely on the membrane transporter Glut1, encoded by Slc2a1, for glucose entry. Slc2a1 is ubiquitously expressed among plaque myeloid cells (Figure 3A,B). However, transcriptomic analysis revealed an enrichment in transcripts related to glycolysis and PPP pathways in macrophages and DCs (Figure 3A,B). Glut1 is solely responsible for glucose entry into macrophages, as its ablation using genetic models demonstrated that other members of this family of transporters cannot substitute its absence [194]. Thus, $Lyz2^{cre} \times Slc2a1^{fl/fl}$ animals have minimal glucose entry associated with decreased levels of many glycolysis and PPP-related metabolites [194]. Compensatory mechanisms led to increased tricarboxylic acid cycle (TCA) metabolites in Glut1-deficent macrophages in comparison to controls [194]. Interestingly, when crossed to atherogenic $Ldlr^{-/-}$ mice, $Lyz2^{cre} \times Slc2a1^{fl/fl} \times Ldlr^{-/-}$ animals had similar plaque size as control mice [194]. Macrophage content, quantified by MOMA2 staining, remained similar as well. However, mice with Glut1-deficient myeloid

cells had an elevated frequency of necrotic core per plaque that paired with a partial deficiency in efferocytosis [194]. This observation was confirmed in another study using the same genetic model [195]. Indeed, efferocytosis triggers a specific metabolic reprogramming of macrophages that relies mainly on glycolysis [195] and lowering glucose concentration, or pharmacological or genetic Glut1-inhibition all efficiently alter macrophage efferocytosis [195]. Glut1 expression is increased following macrophage TLR4 stimulation with LPS to facilitate glucose entry [196], though the relevance of this observation for plaque formation or maintenance requires further investigation. LPS also leads to accelerated glucose flux and increased glycolysis and PPP activation. This is supported by the transcriptional regulation of key enzymes involved in the aforementioned pathways. Thus, LPS increases the expression of two critical enzymes (HK3 and PFKFB3) involved in glycolysis, and this is paralleled by increased pro-inflammatory cytokine production [13]. In plaques, HK3 is found mainly in a population of $Trem2^{+}$ macrophages, while PFKFB3 expression is higher in inflammatory macrophages (Figure 3B). Four HK (hexokinase) isoforms have been identified. Interestingly, HK1 was not enriched in a specific plaque immune subset, while HK2 is highly expressed in inflammatory macrophages (Figure 3B). Again, the biological significance of this observation needs further work. However, macrophage-specific Glut1 overexpression, despite increasing glucose entry and metabolization, failed to generate an increased pro-inflammatory cytokine production [13]. Plaque size, macrophage content and necrotic core area were similar between control and macrophage-Glut1 overexpressing animals [13]. This observation is surprising since increased glucose levels in mice have been associated with a macrophage pro-inflammatory phenotype and disease severity. Taken together, these observations suggest that glucose flux through Glut1 contributes to myeloid cells activation during atherosclerosis, but this is not sufficient to fully explain the pro-inflammatory phenotype of plaque macrophages. Macrophage alternative polarization also requires efficient glucose metabolism [197]. Blocking pyruvate mitochondrial entry and subsequent TCA incorporation leads to decreased ATP production [197]. This is consistent with the role of glucose in TCA cycle activation and ATP generation. Inhibition of the enzyme Acly, playing a key role in Acetyl-CoA generation, prevents macrophage alternative polarization in murine macrophages [198]. The human relevance of this observation was challenged in a recent report using pharmacological inhibitors and genetic approaches [199]. However, whether those pathways affect particularly the metabolic rewiring of a specific subset of plaque resident myeloid cells remains to be defined.

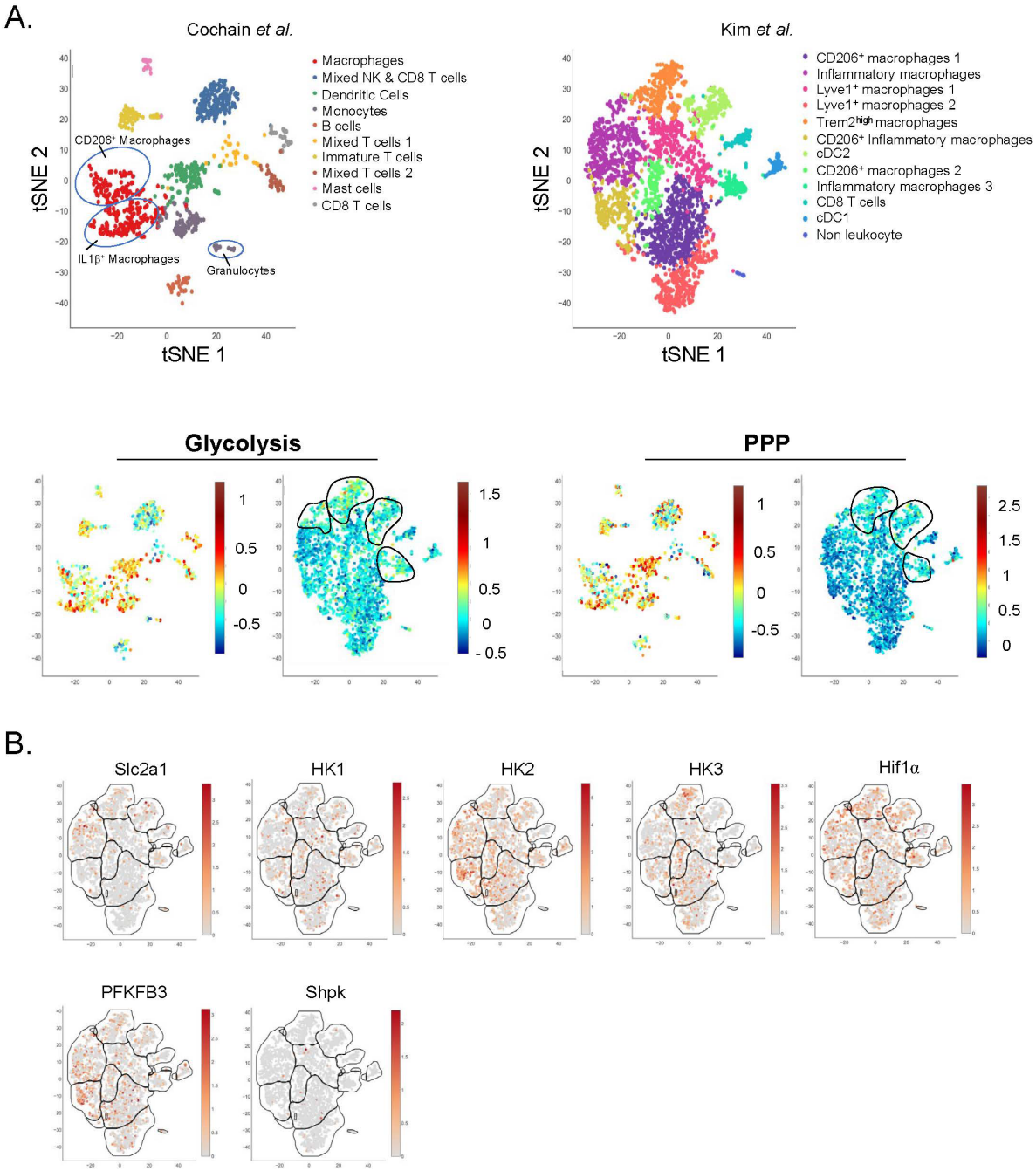


Figure 3. Single-Cell analysis of plaque immune cell glucose metabolism. (**A**,**B**) Single-Cell RNA-Seq of aortic CD45⁺ cells from Ldlr$^{-/-}$ mice fed a HFD for (left) 11 weeks or (right) 12 weeks. Data from (left) Cochain et al. [21] (GSE97310) and (right) Kim et al. [23] (GSM3215435) were analyzed using the Single-Cell Explorer software (Artyomov lab). (**A**) Leukocyte clusters and corresponding KEGG Metabolic Pathway analysis. Glycolysis: KEGG mmu00010. Pentose Phosphate Pathway: KEGG mmu00030. The lists of markers used to identify subsets are in the legend of Figures 1 and 2. (**B**) Expression pattern of genes involved in glucose metabolism (Kim et al. [23] (GSM3215435)).

Interestingly, Folco and colleagues reported no modulations in glucose uptake when stimulating human primary macrophages with pro-inflammatory cytokines [200]. However, glucose uptake was increased in hypoxic conditions, along with increased HK2 expression, while HK1

expression remained unchanged. Immuno-histochemical analysis of human atherosclerotic lesions showed a colocalization of HK2 with the transcription factor HIF-1α (Hypoxia-inducible factor-1), a well-established regulator of glycolysis [200]. Hif-1α mRNA is ubiquitously expressed among plaque resident immune cells, most of which also express HK2 but not HK1, thus supporting these observations (Figure 3B). Advanced plaques contain hypoxic regions due to restricted blood supply and Hif-1a expression was detected in mouse and human plaques [201–204]. Conditional deletion of Hif-1α in myeloid cells ($Lyz2^{cre}$ × $Hif\text{-}1\alpha^{fl/fl}$ mice) didn't impact plaque size [205]. However, when Hif-1α was deleted in CD11c-expressing cells, an increased plaque area was documented, suggesting that this transcription factor mainly operates in CD11c-positive cells that could be DCs or a subset of macrophages (Figure 1B) [205]. $CD11c^{cre}$ × $Hif\text{-}1\alpha^{fl/fl}$ mice displayed increased necrotic core area that might result from defective glucose-driven efferocytosis [205]. However, a recent report demonstrated that $Lyz2^{cre}$ × $Hif\text{-}1\alpha^{fl/fl}$ mice have less plaque lesions when compared to control mice [206]. Surprisingly, Hif-1α deficient mice displayed less apoptotic cells and blunted glucose uptake [206].

LPS also regulates glucose flux into the PPP. LPS decreased the expression of the enzyme Shpk (CARKL) involved in the non-oxidative branch of the PPP [207]. Conversely, IL-4 induced CARKL expression is required for optimal macrophage alternative polarization. CARKL genetic deficiency forces glucose flux into the glycolysis pathway at a level similar to the one seen upon LPS challenge [207]. Shpk expression is not restricted to a selective myeloid cell population in plaques (Figure 3B). In advanced plaque, macrophage local proliferation contributes to plaque growth [208] and one would expect that the PPP pathway, involved in nucleotide generation, is highly activated. This was not yet documented to our knowledge. Interestingly, a recent report demonstrated that hypercholesterolemia suppressed the PPP in macrophages [209]. Whether this mechanism occurs in plaque during atherosclerosis development remains to be tested.

Dendritic cells

Several populations of DCs have been identified in plaques, both in mice and humans (**Table 2 and Figure 1**). However, little is known about the metabolic configuration of plaque resident DCs. Following TLR4 activation with LPS, DCs rapidly undergo a metabolic switch towards glycolysis [210,211]. LPS exposure increases glucose consumption rate and increases Glut1 expression in DCs [210]. This is paralleled by augmented nitric oxide (NO) production that subsequently decreases oxidative phosphorylation (OXPHOS) activity, ATP levels and mitochondrial activity. Consistently, activated DCs show less oxygen consumption rate than resting DCs [210]. Thus, NO seems to play a pivotal role in metabolic regulation [211]. In DCs, endogenous nitric oxide production inhibits OXPHOS and commit those cells to glucose metabolism and aerobic

glycolysis similar to the Warburg effect described in tumor cells [211]. Additionally, LPS-induced NO production contributes to DCs induced death following activation [211]. This glycolytic reprogramming that happens within minutes after TLR stimulation is called the “glycolytic burst” and leads to *de novo* fatty acid synthesis needed for inflammatory cytokine production [212]. In addition, glucose restriction decreases activated-DC maturation, life span and cytokine secretion.

As compared to the rapid increase in glucose flux, Glut1 upregulation in DCs takes hours to build up following TLR stimulation. Therefore, exogenous glucose internalization seems unlikely to be the source required during early DC activation. This lag was recently solved by Thwe and colleagues who showed that intracellular glycogen reserves fuel DCs metabolic demands during early DC activation and that glycogen metabolism is required by these cells to initiate proper immune effector responses [213]. Of note, high glucose concentration increased the oxLDL-uptake capacity of DCs and augmented their IL-6 and IL-12 secretion while decreasing their IL-10 production [214].

Neutrophils

Unlike macrophages and monocytes, little is known about neutrophil metabolic configuration in health and disease. This might be explained by the difficulty to analyze these cells ex vivo. Moreover, single-cell RNA-Seq analysis on neutrophils is rather difficult because these cells typically possess lower number of transcripts when compared to T cells and macrophages. Neutrophils have long been thought to mainly rely on glycolytic metabolism [215], but neutrophils are able to switch from glycolysis to different metabolic pathways such as OXPHOS [216,217]. Increased glycemia favors granulopoiesis and neutrophil release in the blood circulation [191,192]. How modulation of plasma glucose levels impacts on neutrophil chemotaxis, especially their recruitment, retention and survival in the atherosclerotic plaque, is another exciting question.

Amino Acids

In addition to glucose and lipids, amino acids are a source of energy for immune cells. Amino acids are essential metabolites for protein synthesis that act as intermediates in metabolic pathways. Amino acids modulate immune cell functions such as activation, differentiation, proliferation, gene expression, redox status or cytokine production. However, the role of amino acids on immune cell functions during atherosclerosis remains poorly understood.

In vitro studies have demonstrated the impact of glutamine metabolism on macrophage polarization [218,219]. More recently, Tavakoli et al. highlighted increased glutamine accumulation in aortas obtained from $Ldlr^{-/-}$ mice. In this study, the autoradiography shows a non-homogenous glutamine accumulation that diverges according to macrophage activation profile. The combination of 2-deoxyglucose and glutamine accumulation

within the aorta could predict the dominant macrophage polarization profile within the plaque. Indeed, a greater accumulation of glutamine than 2-deoxyglucose supposes a dominant anti-inflammatory population while the opposite indicates a higher content of pro-inflammatory macrophages. This study is one of the first to suggest a role for glutamine on plaque macrophage functions [220].

Besides glutamine, arginine is a key metabolite in vascular function and tone due to its role in the production of nitric oxide (NO). Indeed, arginine is metabolized both by arginase 1 (Arg1) and inducible nitric oxide synthase (iNOS) to produce ornithine and urea or NO respectively. As those two enzymes compete for the same substrate, the use of arginine by Arg1 limits NO production, a macrophage pro-atherogenic factor [221]. In $Ldlr^{-/-}$ mice, microarray analysis showed an increase Arg1 expression in carotid artery during early atherosclerotic lesions. Moreover, Arg1 deficiency promoted NO synthesis upon lipid loading. Hematopoietic Arg1 deletion induced increase foam cell formation in the peritoneum. However, after 10 weeks of western diet, Arg1 deficiency in $Ldlr^{-/-}$ mice had no effect on the plaque size nor on the plaque composition [222]. Similarly, in $Ldlr^{-/-}$ mice deficient for Arg1 specifically in myeloid cells, Yurdagul et al. did not observe any phenotypic difference within the plaque. However, in a regression model, the authors demonstrated defective efferocytosis within the plaque leading to impaired regression, increased necrotic core area and decreased cap thickness. In addition, ornithine produced by Arg1 can be subsequently metabolized to putrescine by ODC (ornithine decarboxylase) [223,224]. Putrescine supplementation improves plaque macrophage efferocytosis leading to reduced lesion and necrotic core size, as well as cap thickness [225]. Conversely, $ApoE^{-/-}$ mice deficient for iNOS in the bone marrow compartment have reduced atheromatous lesions showing that leukocyte mediates the pro-atherogenic effect of iNOS in mice [226].

CONCLUSIONS AND FUTURE DIRECTIONS

The field of immunometabolism is a rapidly expanding one that provides new insights on the role of specific metabolites in immune cells during health and disease. Atherosclerosis is characterized by increased plasma glucose and cholesterol concentrations, and we only recently started to appreciate how precisely these two metabolites impact on plaque resident myeloid cell functions and on their generation from bone marrow-derived precursors. The precise circuits incorporating glucose in macrophages, monocytes, DCs and neutrophils remain to be fully understood. Eventually this might help to apprehend how metabolism supports key functions specific for each population. For example, understanding how metabolism guides monocyte recruitment to plaques as well as their retention or eventual egress will be of significant importance for the field. Regarding macrophages, we recently learned that glucose metabolism sustains one of their key functions: the removal of

apoptotic cells [194,195]. Whether glucose modulates macrophage motility in plaque or their interaction with the extracellular matrix remains to be elucidated. Recent reports demonstrated that DCs cytokine production is tightly regulated by their metabolic configuration [227]. Glucose metabolism regulates DCs migration via regulation of the key chemokine receptor CCR7 [228,229]. Of note, the role of CCR7 during atherosclerosis remains debated with studies reporting that CCR7-deficient mice display smaller [230], similar [231] and increased [232] plaque area. One might wonder whether this mechanism occurs during atherosclerosis as well. Specific metabolic configuration might be required for efficient peptide presentation to conventional T cells via MHC II or lipids to NKT (Natural Killer T) cells via CD1d. Another crucial question is whether the way of metabolite incorporation in myeloid cells affects their intracellular distribution. Apoptotic cell ingestion by myeloid cells leads to internalization of metabolites contained in the dying cell [225]. Thus, the efferocytes need to either incorporate these metabolites into their circuits, store them in specialized compartments or expulse them in the interstitial space where they could be used by neighbor cells.

Single-Cell RNA-Seq moved the field forward toward a better understanding of the immune diversity and functions in atherosclerotic plaque. Nevertheless, the predictions generated via this technique need in detail in situ validation. Recently, an elegant approach was validated to investigate single-cell metabolism in local environment [233]. This new technical advance will be helpful to investigate whether different myeloid cell populations residing in plaque possess a unique enzymatic profile. These analyses could also reveal a zonation in plaque enzyme and metabolite distribution. The field will benefit from our future ability to dose locally metabolites at the scale of the milieu surrounding a cell as well as cellular micro-compartmentalization. This, together with our ability to measure enzymatic activities and at the same scale will certainly make our task of making sense of metabolism an easier one. Our computing ability to integrate all those parameters will also facilitate the large-scale understanding of deciphering how access and competition for nutrients shape immunity and to what extent this can be used as new therapeutic handles.

AUTHOR CONTRIBUTIONS

AG and SI outlined the manuscript. AG, MIS, JM and SI wrote the manuscript. AG generated the figures and tables. RRG, LYC and SI edited the manuscript. All authors approved the final submission of the manuscript.

CONFLICTS OF INTEREST

The authors have no disclosure and conflict of interest to declare.

FUNDING

AG is supported by the French government, through the UCAJedi Investments in the Future projects managed by the National Research Agency (ANR) with the reference number ANR-15-IDEX-01. RRG is supported by Centre National de la Recherche Scientifique (CNRS). LYC is supported by grants from the European Research Council (ERC) consolidator program (ERC2016COG724838). SI is supported by Institut National de la Sante et de la Recherche Medicale (INSERM) and Agence Nationale de la Recherche (ANR-17-CE14-0017-01 and ANR-19-ECVD-0005-01).

REFERENCES

1. Libby P, Lichtman AH, Hansson GK. Immune effector mechanisms implicated in atherosclerosis: from mice to humans. Immunity. 2013;38(6):1092-104.
2. Kobiyama K, Ley K. Atherosclerosis. Circ Res. 2018;123(10):1118-20.
3. Fredrickson DS, Lees RS. A System for Phenotyping Hyperlipoproteinemia. Circulation. 1965;31:321-7.
4. Dawber TR, Moore FE, Mann GV. Coronary heart disease in the Framingham study. Am J Public Health Nations Health. 1957;47(4 Pt 2):4-24.
5. Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. Cell. 2011;145(3):341-55.
6. Otsuka F, Joner M, Prati F, Virmani R, Narula J. Clinical classification of plaque morphology in coronary disease. Nat Rev Cardiol. 2014;11(7):379-89.
7. Tabas I. Heart disease: Death-defying plaque cells. Nature. 2016;536(7614):32-3.
8. Davies MJ, Richardson PD, Woolf N, Katz DR, Mann J. Risk of thrombosis in human atherosclerotic plaques: role of extracellular lipid, macrophage, and smooth muscle cell content. Br Heart J. 1993;69(5):377-81.
9. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. Arterioscler Thromb Vasc Biol. 2000;20(5):1262-75.
10. Guilliams M, Ginhoux F, Jakubzick C, Naik SH, Onai N, Schraml BU, et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. Nat Rev Immunol. 2014;14(8):571-8.
11. Gautier EL, Shay T, Miller J, Greter M, Jakubzick C, Ivanov S, et al. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. Nat Immunol. 2012;13(11):1118-28.
12. Tabas I, Bornfeldt KE. Intracellular and Intercellular Aspects of Macrophage Immunometabolism in Atherosclerosis. Circ Res. 2020;126(9):1209-27.
13. Nishizawa T, Kanter JE, Kramer F, Barnhart S, Shen X, Vivekanandan-Giri A, et al. Testing the role of myeloid cell glucose flux in inflammation and atherosclerosis. Cell Rep. 2014;7(2):356-65.
14. Shirai T, Nazarewicz RR, Wallis BB, Yanes RE, Watanabe R, Hilhorst M, et al. The glycolytic enzyme PKM2 bridges metabolic and inflammatory dysfunction in coronary artery disease. J Exp Med. 2016;213(3):337-54.

15. Tomas L, Edsfeldt A, Mollet IG, Perisic Matic L, Prehn C, Adamski J, et al. Altered metabolism distinguishes high-risk from stable carotid atherosclerotic plaques. Eur Heart J. 2018;39(24):2301-10.
16. Jonasson L, Holm J, Skalli O, Bondjers G, Hansson GK. Regional accumulations of T cells, macrophages, and smooth muscle cells in the human atherosclerotic plaque. Arteriosclerosis. 1986;6(2):131-8.
17. Koltsova EK, Garcia Z, Chodaczek G, Landau M, McArdle S, Scott SR, et al. Dynamic T cell-APC interactions sustain chronic inflammation in atherosclerosis. J Clin Invest. 2012;122(9):3114-26.
18. Lin JD, Nishi H, Poles J, Niu X, McCauley C, Rahman K, et al. Single-cell analysis of fate-mapped macrophages reveals heterogeneity, including stem-like properties, during atherosclerosis progression and regression. JCI Insight. 2019;4(4):e124574.
19. Williams JW, Winkels H, Durant CP, Zaitsev K, Ghosheh Y, Ley K. Single Cell RNA Sequencing in Atherosclerosis Research. Circ Res. 2020;126(9):1112-26.
20. Fernandez DM, Rahman AH, Fernandez NF, Chudnovskiy A, Amir ED, Amadori L, et al. Single-cell immune landscape of human atherosclerotic plaques. Nat Med. 2019;25(10):1576-88.
21. Cochain C, Vafadarnejad E, Arampatzi P, Pelisek J, Winkels H, Ley K, et al. Single-Cell RNA-Seq Reveals the Transcriptional Landscape and Heterogeneity of Aortic Macrophages in Murine Atherosclerosis. Circ Res. 2018;122(12):1661-74.
22. Winkels H, Ehinger E, Vassallo M, Buscher K, Dinh HQ, Kobiyama K, et al. Atlas of the Immune Cell Repertoire in Mouse Atherosclerosis Defined by Single-Cell RNA-Sequencing and Mass Cytometry. Circ Res. 2018;122(12):1675-88.
23. Kim K, Shim D, Lee JS, Zaitsev K, Williams JW, Kim KW, et al. Transcriptome Analysis Reveals Nonfoamy Rather Than Foamy Plaque Macrophages Are Proinflammatory in Atherosclerotic Murine Models. Circ Res. 2018;123(10):1127-42.
24. Cole JE, Park I, Ahern DJ, Kassiteridi C, Danso Abeam D, Goddard ME, et al. Immune cell census in murine atherosclerosis: cytometry by time of flight illuminates vascular myeloid cell diversity. Cardiovasc Res. 2018;114(10):1360-71.
25. Kalluri AS, Vellarikkal SK, Edelman ER, Nguyen L, Subramanian A, Ellinor PT, et al. Single-Cell Analysis of the Normal Mouse Aorta Reveals Functionally Distinct Endothelial Cell Populations. Circulation. 2019;140(2):147-63.
26. Zernecke A, Winkels H, Cochain C, Williams JW, Wolf D, Soehnlein O, et al. Meta-Analysis of Leukocyte Diversity in Atherosclerotic Mouse Aortas. Circ Res. 2020;127(3):402-26.
27. Wolf AA, Yanez A, Barman PK, Goodridge HS. The Ontogeny of Monocyte Subsets. Front Immunol. 2019;10:1642.
28. Jakubzick CV, Randolph GJ, Henson PM. Monocyte differentiation and antigen-presenting functions. Nat Rev Immunol. 2017;17(6):349-62.
29. Dai XM, Ryan GR, Hapel AJ, Dominguez MG, Russell RG, Kapp S, et al. Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in

osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects. Blood. 2002;99(1):111-20.
30. Guilliams M, Thierry GR, Bonnardel J, Bajenoff M. Establishment and Maintenance of the Macrophage Niche. Immunity. 2020;52(3):434-51.
31. Friedman GD, Klatsky AL, Siegelaub AB. The leukocyte count as a predictor of myocardial infarction. N Engl J Med. 1974;290(23):1275-8.
32. Barron HV, Cannon CP, Murphy SA, Braunwald E, Gibson CM. Association between white blood cell count, epicardial blood flow, myocardial perfusion, and clinical outcomes in the setting of acute myocardial infarction: a thrombolysis in myocardial infarction 10 substudy. Circulation. 2000;102(19):2329-34.
33. Cannon CP, McCabe CH, Wilcox RG, Bentley JH, Braunwald E. Association of white blood cell count with increased mortality in acute myocardial infarction and unstable angina pectoris. OPUS-TIMI 16 Investigators. Am J Cardiol. 2001;87(5):636-9,A10.
34. Averill LE, Meagher RC, Gerrity RG. Enhanced monocyte progenitor cell proliferation in bone marrow of hyperlipemic swine. Am J Pathol. 1989;135(2):369-77.
35. Feldman DL, Mogelesky TC, Liptak BF, Gerrity RG. Leukocytosis in rabbits with diet-induced atherosclerosis. Arterioscler Thromb. 1991;11(4):985-94.
36. Lessner SM, Prado HL, Waller EK, Galis ZS. Atherosclerotic lesions grow through recruitment and proliferation of circulating monocytes in a murine model. Am J Pathol. 2002;160(6):2145-55.
37. Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. Immunity. 2003;19(1):71-82.
38. Sunderkotter C, Nikolic T, Dillon MJ, Van Rooijen N, Stehling M, Drevets DA, et al. Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. J Immunol. 2004;172(7):4410-7.
39. Chong SZ, Evrard M, Devi S, Chen J, Lim JY, See P, et al. CXCR4 identifies transitional bone marrow premonocytes that replenish the mature monocyte pool for peripheral responses. J Exp Med. 2016;213(11):2293-314.
40. Hanna RN, Carlin LM, Hubbeling HG, Nackiewicz D, Green AM, Punt JA, et al. The transcription factor NR4A1 (Nur77) controls bone marrow differentiation and the survival of Ly6C- monocytes. Nat Immunol. 2011;12(8):778-85.
41. Auffray C, Fogg D, Garfa M, Elain G, Join-Lambert O, Kayal S, et al. Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. Science. 2007;317(5838):666-70.
42. Carlin LM, Stamatiades EG, Auffray C, Hanna RN, Glover L, Vizcay-Barrena G, et al. Nr4a1-dependent Ly6C(low) monocytes monitor endothelial cells and orchestrate their disposal. Cell. 2013;153(2):362-75.
43. Weber C, Belge KU, von Hundelshausen P, Draude G, Steppich B, Mack M, et al. Differential chemokine receptor expression and function in human monocyte subpopulations. J Leukoc Biol. 2000;67(5):699-704.
44. Palframan RT, Jung S, Cheng G, Weninger W, Luo Y, Dorf M, et al. Inflammatory chemokine transport and presentation in HEV: a remote

control mechanism for monocyte recruitment to lymph nodes in inflamed tissues. J Exp Med. 2001;194(9):1361-73.

45. Dyer DP, Medina-Ruiz L, Bartolini R, Schuette F, Hughes CE, Pallas K, et al. Chemokine Receptor Redundancy and Specificity Are Context Dependent. Immunity. 2019;50(2):378-89.e5.
46. Swirski FK, Libby P, Aikawa E, Alcaide P, Luscinskas FW, Weissleder R, et al. Ly-6Chi monocytes dominate hypercholesterolemia-associated monocytosis and give rise to macrophages in atheromata. J Clin Invest. 2007;117(1):195-205.
47. Tacke F, Alvarez D, Kaplan TJ, Jakubzick C, Spanbroek R, Llodra J, et al. Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. J Clin Invest. 2007;117(1):185-94.
48. Imhof BA, Aurrand-Lions M. Adhesion mechanisms regulating the migration of monocytes. Nat Rev Immunol. 2004;4(6):432-44.
49. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat Rev Immunol. 2007;7(9):678-89.
50. Verweij SL, Duivenvoorden R, Stiekema LCA, Nurmohamed NS, van der Valk FM, Versloot M, et al. CCR2 expression on circulating monocytes is associated with arterial wall inflammation assessed by 18F-FDG PET/CT in patients at risk for cardiovascular disease. Cardiovasc Res. 2018;114(3):468-75.
51. Yoshida H, Hayashi S, Kunisada T, Ogawa M, Nishikawa S, Okamura H, et al. The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. Nature. 1990;345(6274):442-4.
52. Qiao JH, Tripathi J, Mishra NK, Cai Y, Tripathi S, Wang XP, et al. Role of macrophage colony-stimulating factor in atherosclerosis: studies of osteopetrotic mice. Am J Pathol. 1997;150(5):1687-99.
53. Smith JD, Trogan E, Ginsberg M, Grigaux C, Tian J, Miyata M. Decreased atherosclerosis in mice deficient in both macrophage colony-stimulating factor (op) and apolipoprotein E. Proc Natl Acad Sci U S A. 1995;92(18):8264-8.
54. Rajavashisth T, Qiao JH, Tripathi S, Tripathi J, Mishra N, Hua M, et al. Heterozygous osteopetrotic (op) mutation reduces atherosclerosis in LDL receptor- deficient mice. J Clin Invest. 1998;101(12):2702-10.
55. Boring L, Gosling J, Cleary M, Charo IF. Decreased lesion formation in CCR2$^{-/-}$ mice reveals a role for chemokines in the initiation of atherosclerosis. Nature. 1998;394(6696):894-7.
56. Gu L, Okada Y, Clinton SK, Gerard C, Sukhova GK, Libby P, et al. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. Mol Cell. 1998;2(2):275-81.
57. Ingersoll MA, Spanbroek R, Lottaz C, Gautier EL, Frankenberger M, Hoffmann R, et al. Comparison of gene expression profiles between human and mouse monocyte subsets. Blood. 2010;115(3):e10-9.
58. Ait-Oufella H, Taleb S, Mallat Z, Tedgui A. Recent advances on the role of cytokines in atherosclerosis. Arterioscler Thromb Vasc Biol. 2011;31(5):969-79.

59. Galea J, Armstrong J, Gadsdon P, Holden H, Francis SE, Holt CM. Interleukin-1 beta in coronary arteries of patients with ischemic heart disease. Arterioscler Thromb Vasc Biol. 1996;16(8):1000-6.
60. Kirii H, Niwa T, Yamada Y, Wada H, Saito K, Iwakura Y, et al. Lack of interleukin-1beta decreases the severity of atherosclerosis in ApoE-deficient mice. Arterioscler Thromb Vasc Biol. 2003;23(4):656-60.
61. Isoda K, Sawada S, Ishigami N, Matsuki T, Miyazaki K, Kusuhara M, et al. Lack of interleukin-1 receptor antagonist modulates plaque composition in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol. 2004;24(6):1068-73.
62. Gomez D, Baylis RA, Durgin BG, Newman AAC, Alencar GF, Mahan S, et al. Interleukin-1beta has atheroprotective effects in advanced atherosclerotic lesions of mice. Nat Med. 2018;24(9):1418-29.
63. Ridker PM, MacFadyen JG, Everett BM, Libby P, Thuren T, Glynn RJ, et al. Relationship of C-reactive protein reduction to cardiovascular event reduction following treatment with canakinumab: a secondary analysis from the CANTOS randomised controlled trial. Lancet. 2018;391(10118):319-28.
64. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, et al. Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. N Engl J Med. 2017;377(12):1119-31.
65. Doran AC, Yurdagul A Jr, Tabas I. Efferocytosis in health and disease. Nat Rev Immunol. 2020;20(4):254-67.
66. Bianconi E, Piovesan A, Facchin F, Beraudi A, Casadei R, Frabetti F, et al. An estimation of the number of cells in the human body. Ann Hum Biol. 2013;40(6):463-71.
67. Wolf Y, Boura-Halfon S, Cortese N, Haimon Z, Sar Shalom H, Kuperman Y, et al. Brown-adipose-tissue macrophages control tissue innervation and homeostatic energy expenditure. Nat Immunol. 2017;18(6):665-74.
68. Kohyama M, Ise W, Edelson BT, Wilker PR, Hildner K, Mejia C, et al. Role for Spi-C in the development of red pulp macrophages and splenic iron homeostasis. Nature. 2009;457(7227):318-21.
69. Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science. 2012;336(6077):86-90.
70. Gomez Perdiguero E, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. Nature. 2015;518(7540):547-51.
71. Guilliams M, De Kleer I, Henri S, Post S, Vanhoutte L, De Prijck S, et al. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. J Exp Med. 2013;210(10):1977-92.
72. Bain CC, Mowat AM. Macrophages in intestinal homeostasis and inflammation. Immunol Rev. 2014;260(1):102-17.
73. Epelman S, Lavine KJ, Beaudin AE, Sojka DK, Carrero JA, Calderon B, et al. Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. Immunity. 2014;40(1):91-104.

74. Silva HM, Bafica A, Rodrigues-Luiz GF, Chi J, Santos PDA, Reis BS, et al. Vasculature-associated fat macrophages readily adapt to inflammatory and metabolic challenges. J Exp Med. 2019;216(4):786-806.
75. Wang Y, Szretter KJ, Vermi W, Gilfillan S, Rossini C, Cella M, et al. IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. Nat Immunol. 2012;13(8):753-60.
76. Ivanov S, Gallerand A, Gros M, Stunault MI, Merlin J, Vaillant N, et al. Mesothelial cell CSF1 sustains peritoneal macrophage proliferation. Eur J Immunol. 2019;49(11):2012-8.
77. Chakarov S, Lim HY, Tan L, Lim SY, See P, Lum J, et al. Two distinct interstitial macrophage populations coexist across tissues in specific subtissular niches. Science. 2019;363(6432):eaau0964.
78. Cochain C, Saliba AE, Zernecke A. Letter by Cochain et al Regarding Article, "Transcriptome Analysis Reveals Nonfoamy Rather Than Foamy Plaque Macrophages Are Proinflammatory in Atherosclerotic Murine Models". Circ Res. 2018;123(11):e48-9.
79. Kim K, Choi JH. Response by Kim and Choi to Letter Regarding Article, "Transcriptome Analysis Reveals Nonfoamy Rather Than Foamy Plaque Macrophages Are Proinflammatory in Atherosclerotic Murine Models". Circ Res. 2018;123(11):e50.
80. Satpathy AT, Kc W, Albring JC, Edelson BT, Kretzer NM, Bhattacharya D, et al. Zbtb46 expression distinguishes classical dendritic cells and their committed progenitors from other immune lineages. J Exp Med. 2012;209(6):1135-52.
81. Meredith MM, Liu K, Darrasse-Jeze G, Kamphorst AO, Schreiber HA, Guermonprez P, et al. Expression of the zinc finger transcription factor zDC (Zbtb46, Btbd4) defines the classical dendritic cell lineage. J Exp Med. 2012;209(6):1153-65.
82. Murphy TL, Grajales-Reyes GE, Wu X, Tussiwand R, Briseno CG, Iwata A, et al. Transcriptional Control of Dendritic Cell Development. Annu Rev Immunol. 2016;34:93-119.
83. Doring Y, Zernecke A. Plasmacytoid dendritic cells in atherosclerosis. Front Physiol. 2012;3:230.
84. Jensen J, Vazquez-Torres A, Balish E. Poly(I.C)-induced interferons enhance susceptibility of SCID mice to systemic candidiasis. Infect Immun. 1992;60(11):4549-57.
85. Thacker SG, Zhao W, Smith CK, Luo W, Wang H, Vivekanandan-Giri A, et al. Type I interferons modulate vascular function, repair, thrombosis, and plaque progression in murine models of lupus and atherosclerosis. Arthritis Rheum. 2012;64(9):2975-85.
86. Ait-Oufella H, Sage AP, Mallat Z, Tedgui A. Adaptive (T and B cells) immunity and control by dendritic cells in atherosclerosis. Circ Res. 2014;114(10):1640-60.
87. Weber C, Meiler S, Doring Y, Koch M, Drechsler M, Megens RT, et al. CCL17-expressing dendritic cells drive atherosclerosis by restraining regulatory T cell homeostasis in mice. J Clin Invest. 2011;121(7):2898-910.
88. Kimura T, Kobiyama K, Winkels H, Tse K, Miller J, Vassallo M, et al. Regulatory CD4(+) T Cells Recognize Major Histocompatibility Complex Class II Molecule-

Restricted Peptide Epitopes of Apolipoprotein B. Circulation. 2018;138(11):1130-43.

89. Kimura T, Tse K, McArdle S, Gerhardt T, Miller J, Mikulski Z, et al. Atheroprotective vaccination with MHC-II-restricted ApoB peptides induces peritoneal IL-10-producing CD4 T cells. Am J Physiol Heart Circ Physiol. 2017;312(4):H781-90.
90. Tse K, Gonen A, Sidney J, Ouyang H, Witztum JL, Sette A, et al. Atheroprotective Vaccination with MHC-II Restricted Peptides from ApoB-100. Front Immunol. 2013;4:493.
91. Lin Z, Qian S, Gong Y, Ren J, Zhao L, Wang D, et al. Deep sequencing of the T cell receptor beta repertoire reveals signature patterns and clonal drift in atherosclerotic plaques and patients. Oncotarget. 2017;8(59):99312-22.
92. Paulsson G, Zhou X, Tornquist E, Hansson GK. Oligoclonal T cell expansions in atherosclerotic lesions of apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol. 2000;20(1):10-7.
93. Buono C, Pang H, Uchida Y, Libby P, Sharpe AH, Lichtman AH. B7-1/B7-2 costimulation regulates plaque antigen-specific T-cell responses and atherogenesis in low-density lipoprotein receptor-deficient mice. Circulation. 2004;109(16):2009-15.
94. Haddad Y, Lahoute C, Clement M, Laurans L, Metghalchi S, Zeboudj L, et al. The Dendritic Cell Receptor DNGR-1 Promotes the Development of Atherosclerosis in Mice. Circ Res. 2017;121(3):234-43.
95. Nathan C. Neutrophils and immunity: challenges and opportunities. Nat Rev Immunol. 2006;6(3):173-82.
96. Doring Y, Drechsler M, Wantha S, Kemmerich K, Lievens D, Vijayan S, et al. Lack of neutrophil-derived CRAMP reduces atherosclerosis in mice. Circ Res. 2012;110(8):1052-6.
97. Zernecke A, Bot I, Djalali-Talab Y, Shagdarsuren E, Bidzhekov K, Meiler S, et al. Protective role of CXC receptor 4/CXC ligand 12 unveils the importance of neutrophils in atherosclerosis. Circ Res. 2008;102(2):209-17.
98. Naruko T, Ueda M, Haze K, van der Wal AC, van der Loos CM, Itoh A, et al. Neutrophil infiltration of culprit lesions in acute coronary syndromes. Circulation. 2002;106(23):2894-900.
99. Rotzius P, Thams S, Soehnlein O, Kenne E, Tseng CN, Bjorkstrom NK, et al. Distinct infiltration of neutrophils in lesion shoulders in ApoE$^{-/-}$ mice. Am J Pathol. 2010;177(1):493-500.
100. Yang, Han Z, Oppenheim JJ. Alarmins and immunity. Immunol Rev. 2017;280(1):41-56.
101. Pruenster M, Kurz AR, Chung KJ, Cao-Ehlker X, Bieber S, Nussbaum CF, et al. Extracellular MRP8/14 is a regulator of beta2 integrin-dependent neutrophil slow rolling and adhesion. Nat Commun. 2015;6:6915.
102. Chen Q, Jin Y, Zhang K, Li H, Chen W, Meng G, et al. Alarmin HNP-1 promotes pyroptosis and IL-1beta release through different roles of NLRP3 inflammasome via P2X7 in LPS-primed macrophages. Innate Immun. 2014;20(3):290-300.

103. Willingham SB, Allen IC, Bergstralh DT, Brickey WJ, Huang MT, Taxman DJ, et al. NLRP3 (NALP3, Cryopyrin) facilitates in vivo caspase-1 activation, necrosis, and HMGB1 release via inflammasome-dependent and -independent pathways. J Immunol. 2009;183(3):2008-15.
104. Kahlenberg JM, Carmona-Rivera C, Smith CK, Kaplan MJ. Neutrophil extracellular trap-associated protein activation of the NLRP3 inflammasome is enhanced in lupus macrophages. J Immunol. 2013;190(3):1217-26.
105. Soehnlein O, Zernecke A, Eriksson EE, Rothfuchs AG, Pham CT, Herwald H, et al. Neutrophil secretion products pave the way for inflammatory monocytes. Blood. 2008;112(4):1461-71.
106. Qi H, Yang S, Zhang L. Neutrophil Extracellular Traps and Endothelial Dysfunction in Atherosclerosis and Thrombosis. Front Immunol. 2017;8:928.
107. Schuster S, Hurrell B, Tacchini-Cottier F. Crosstalk between neutrophils and dendritic cells: a context-dependent process. J Leukoc Biol. 2013;94(4):671-5.
108. Narula J, Kolodgie FD, Virmani R. Apoptosis and cardiomyopathy. Curr Opin Cardiol. 2000;15(3):183-8.
109. Denny MF, Yalavarthi S, Zhao W, Thacker SG, Anderson M, Sandy AR, et al. A distinct subset of proinflammatory neutrophils isolated from patients with systemic lupus erythematosus induces vascular damage and synthesizes type I IFNs. J Immunol. 2010;184(6):3284-97.
110. Chavez-Sanchez L, Espinosa-Luna JE, Chavez-Rueda K, Legorreta-Haquet MV, Montoya-Diaz E, Blanco-Favela F. Innate immune system cells in atherosclerosis. Arch Med Res. 2014;45(1):1-14.
111. van Leeuwen M, Gijbels MJ, Duijvestijn A, Smook M, van de Gaar MJ, Heeringa P, et al. Accumulation of myeloperoxidase-positive neutrophils in atherosclerotic lesions in LDLR$^{-/-}$ mice. Arterioscler Thromb Vasc Biol. 2008;28(1):84-9.
112. Aratani Y. Myeloperoxidase: Its role for host defense, inflammation, and neutrophil function. Arch Biochem Biophys. 2018;640:47-52.
113. Podrez EA, Schmitt D, Hoff HF, Hazen SL. Myeloperoxidase-generated reactive nitrogen species convert LDL into an atherogenic form in vitro. J Clin Invest. 1999;103(11):1547-60.
114. Khan MA, Palaniyar N. Transcriptional firing helps to drive NETosis. Sci Rep. 2017;7:41749.
115. Doring Y, Libby P, Soehnlein O. Neutrophil Extracellular Traps Participate in Cardiovascular Diseases: Recent Experimental and Clinical Insights. Circ Res. 2020;126(9):1228-41.
116. Sriranjan RS, Tarkin JM, Evans NR, Le EPV, Chowdhury MM, Rudd JHF. Atherosclerosis imaging using PET: Insights and applications. Br J Pharmacol. 2019. doi: 10.1111/bph.14868
117. Yamashita A, Zhao Y, Matsuura Y, Yamasaki K, Moriguchi-Goto S, Sugita C, et al. Increased metabolite levels of glycolysis and pentose phosphate pathway in rabbit atherosclerotic arteries and hypoxic macrophage. PLoS One. 2014;9(1):e86426.

118. Yvan-Charvet L, Pagler T, Gautier EL, Avagyan S, Siry RL, Han S, et al. ATP-binding cassette transporters and HDL suppress hematopoietic stem cell proliferation. Science. 2010;328(5986):1689-93.
119. Murphy AJ, Akhtari M, Tolani S, Pagler T, Bijl N, Kuo CL, et al. ApoE regulates hematopoietic stem cell proliferation, monocytosis, and monocyte accumulation in atherosclerotic lesions in mice. J Clin Invest. 2011;121(10):4138-49.
120. Wang M, Subramanian M, Abramowicz S, Murphy AJ, Gonen A, Witztum J, et al. Interleukin-3/granulocyte macrophage colony-stimulating factor receptor promotes stem cell expansion, monocytosis, and atheroma macrophage burden in mice with hematopoietic ApoE deficiency. Arterioscler Thromb Vasc Biol. 2014;34(5):976-84.
121. Morgan PK, Fang L, Lancaster GI, Murphy AJ. Hematopoiesis is regulated by cholesterol efflux pathways and lipid rafts: connections with cardiovascular diseases. J Lipid Res. 2020;61(5):667-75.
122. Hermetet F, Buffiere A, Aznague A, Pais de Barros JP, Bastie JN, Delva L, et al. High-fat diet disturbs lipid raft/TGF-beta signaling-mediated maintenance of hematopoietic stem cells in mouse bone marrow. Nat Commun. 2019;10(1):523.
123. Babaev VR, Runner RP, Fan D, Ding L, Zhang Y, Tao H, et al. Macrophage Mal1 deficiency suppresses atherosclerosis in low-density lipoprotein receptor-null mice by activating peroxisome proliferator-activated receptor-gamma-regulated genes. Arterioscler Thromb Vasc Biol. 2011;31(6):1283-90.
124. Takahashi M, Yagyu H, Tazoe F, Nagashima S, Ohshiro T, Okada K, et al. Macrophage lipoprotein lipase modulates the development of atherosclerosis but not adiposity. J Lipid Res. 2013;54(4):1124-34.
125. Chang CL, Garcia-Arcos I, Nyren R, Olivecrona G, Kim JY, Hu Y, et al. Lipoprotein Lipase Deficiency Impairs Bone Marrow Myelopoiesis and Reduces Circulating Monocyte Levels. Arterioscler Thromb Vasc Biol. 2018;38(3):509-19.
126. Jordan S, Tung N, Casanova-Acebes M, Chang C, Cantoni C, Zhang D, et al. Dietary Intake Regulates the Circulating Inflammatory Monocyte Pool. Cell. 2019;178(5):1102-14.e17.
127. Fowler S, Shio H, Haley NJ. Characterization of lipid-laden aortic cells from cholesterol-fed rabbits. IV. Investigation of macrophage-like properties of aortic cell populations. Lab Invest. 1979;41(4):372-8.
128. Brown MS, Goldstein JL. Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. Annu Rev Biochem. 1983;52:223-61.
129. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. Science. 1986;232(4746):34-47.
130. Suzuki H, Kurihara Y, Takeya M, Kamada N, Kataoka M, Jishage K, et al. A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection. Nature. 1997;386(6622):292-6.
131. Kunjathoor VV, Febbraio M, Podrez EA, Moore KJ, Andersson L, Koehn S, et al. Scavenger receptors class A-I/II and CD36 are the principal receptors

responsible for the uptake of modified low density lipoprotein leading to lipid loading in macrophages. J Biol Chem. 2002;277(51):49982-8.

132. Yvan-Charvet L, Ranalletta M, Wang N, Han S, Terasaka N, Li R, et al. Combined deficiency of ABCA1 and ABCG1 promotes foam cell accumulation and accelerates atherosclerosis in mice. J Clin Invest. 2007;117(12):3900-8.
133. Accad M, Smith SJ, Newland DL, Sanan DA, King LE Jr, Linton MF, et al. Massive xanthomatosis and altered composition of atherosclerotic lesions in hyperlipidemic mice lacking acyl CoA:cholesterol acyltransferase 1. J Clin Invest. 2000;105(6):711-9.
134. Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. Nature. 2010;464(7293):1357-61.
135. Schuster GU, Parini P, Wang L, Alberti S, Steffensen KR, Hansson GK, et al. Accumulation of foam cells in liver X receptor-deficient mice. Circulation. 2002;106(9):1147-53.
136. Larrede S, Quinn CM, Jessup W, Frisdal E, Olivier M, Hsieh V, et al. Stimulation of cholesterol efflux by LXR agonists in cholesterol-loaded human macrophages is ABCA1-dependent but ABCG1-independent. Arterioscler Thromb Vasc Biol. 2009;29(11):1930-6.
137. Calkin AC, Tontonoz P. Liver × receptor signaling pathways and atherosclerosis. Arterioscler Thromb Vasc Biol. 2010;30(8):1513-8.
138. Bischoff ED, Daige CL, Petrowski M, Dedman H, Pattison J, Juliano J, et al. Non-redundant roles for LXRalpha and LXRbeta in atherosclerosis susceptibility in low density lipoprotein receptor knockout mice. J Lipid Res. 2010;51(5):900-6.
139. Westerterp M, Murphy AJ, Wang M, Pagler TA, Vengrenyuk Y, Kappus MS, et al. Deficiency of ATP-binding cassette transporters A1 and G1 in macrophages increases inflammation and accelerates atherosclerosis in mice. Circ Res. 2013;112(11):1456-65.
140. Chinetti-Gbaguidi G, Baron M, Bouhlel MA, Vanhoutte J, Copin C, Sebti Y, et al. Human atherosclerotic plaque alternative macrophages display low cholesterol handling but high phagocytosis because of distinct activities of the PPARgamma and LXRalpha pathways. Circ Res. 2011;108(8):985-95.
141. Vats D, Mukundan L, Odegaard JI, Zhang L, Smith KL, Morel CR, et al. Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. Cell Metab. 2006;4(1):13-24.
142. Chinetti G, Lestavel S, Bocher V, Remaley AT, Neve B, Torra IP, et al. PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. Nat Med. 2001;7(1):53-8.
143. Chawla A, Boisvert WA, Lee CH, Laffitte BA, Barak Y, Joseph SB, et al. A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. Mol Cell. 2001;7(1):161-71.
144. Li AC, Binder CJ, Gutierrez A, Brown KK, Plotkin CR, Pattison JW, et al. Differential inhibition of macrophage foam-cell formation and atherosclerosis in mice by PPARalpha, beta/delta, and gamma. J Clin Invest. 2004;114(11):1564-76.

145. Babaev VR, Ishiguro H, Ding L, Yancey PG, Dove DE, Kovacs WJ, et al. Macrophage expression of peroxisome proliferator-activated receptor-alpha reduces atherosclerosis in low-density lipoprotein receptor-deficient mice. Circulation. 2007;116(12):1404-12.
146. Rigamonti E, Chinetti-Gbaguidi G, Staels B. Regulation of macrophage functions by PPAR-alpha, PPAR-gamma, and LXRs in mice and men. Arterioscler Thromb Vasc Biol. 2008;28(6):1050-9.
147. Dubois V, Eeckhoute J, Lefebvre P, Staels B. Distinct but complementary contributions of PPAR isotypes to energy homeostasis. J Clin Invest. 2017;127(4):1202-14.
148. Viaud M, Ivanov S, Vujic N, Duta-Mare M, Aira LE, Barouillet T, et al. Lysosomal Cholesterol Hydrolysis Couples Efferocytosis to Anti-Inflammatory Oxysterol Production. Circ Res. 2018;122(10):1369-84.
149. Wild PS, Zeller T, Schillert A, Szymczak S, Sinning CR, Deiseroth A, et al. A genome-wide association study identifies LIPA as a susceptibility gene for coronary artery disease. Circ Cardiovasc Genet. 2011;4(4):403-12.
150. Evans TD, Zhang X, Clark RE, Alisio A, Song E, Zhang H, et al. Functional Characterization of LIPA (Lysosomal Acid Lipase) Variants Associated With Coronary Artery Disease. Arterioscler Thromb Vasc Biol. 2019;39(12):2480-91.
151. Horkko S, Bird DA, Miller E, Itabe H, Leitinger N, Subbanagounder G, et al. Monoclonal autoantibodies specific for oxidized phospholipids or oxidized phospholipid-protein adducts inhibit macrophage uptake of oxidized low-density lipoproteins. J Clin Invest. 1999;103(1):117-28.
152. Tsimikas S, Miyanohara A, Hartvigsen K, Merki E, Shaw PX, Chou MY, et al. Human oxidation-specific antibodies reduce foam cell formation and atherosclerosis progression. J Am Coll Cardiol. 2011;58(16):1715-27.
153. Que X, Hung MY, Yeang C, Gonen A, Prohaska TA, Sun X, et al. Oxidized phospholipids are proinflammatory and proatherogenic in hypercholesterolaemic mice. Nature. 2018;558(7709):301-6.
154. Palinski W, Tangirala RK, Miller E, Young SG, Witztum JL. Increased autoantibody titers against epitopes of oxidized LDL in LDL receptor-deficient mice with increased atherosclerosis. Arterioscler Thromb Vasc Biol. 1995;15(10):1569-76.
155. Harmon EY, Fronhofer V, 3rd, Keller RS, Feustel PJ, Zhu X, Xu H, et al. Anti-inflammatory immune skewing is atheroprotective: Apoe$^{-/-}$FcgammaRIIb$^{-/-}$ mice develop fibrous carotid plaques. J Am Heart Assoc. 2014;3(6):e001232.
156. Pagano S, Magenta A, D'Agostino M, Martino F, Barilla F, Satta N, et al. Anti-ApoA-1 IgGs in Familial Hypercholesterolemia Display Paradoxical Associations with Lipid Profile and Promote Foam Cell Formation. J Clin Med. 2019;8(12):2035.
157. van den Berg VJ, Vroegindewey MM, Kardys I, Boersma E, Haskard D, Hartley A, et al. Anti-Oxidized LDL Antibodies and Coronary Artery Disease: A Systematic Review. Antioxidants (Basel). 2019;8(10):484.
158. van Dierendonck X, de la Rosa Rodriguez MA, Georgiadi A, Mattijssen F, Dijk W, van Weeghel M, et al. HILPDA Uncouples Lipid Droplet Accumulation in

Adipose Tissue Macrophages from Inflammation and Metabolic Dysregulation. Cell Rep. 2020;30(6):1811-22.e6.

159. Maier A, Wu H, Cordasic N, Oefner P, Dietel B, Thiele C, et al. Hypoxia-inducible protein 2 Hig2/Hilpda mediates neutral lipid accumulation in macrophages and contributes to atherosclerosis in apolipoprotein E-deficient mice. FASEB J. 2017;31(11):4971-84.
160. Gomez I, Foudi N, Longrois D, Norel X. The role of prostaglandin E2 in human vascular inflammation. Prostaglandins Leukot Essent Fatty Acids. 2013;89(2-3):55-63.
161. Spann NJ, Garmire LX, McDonald JG, Myers DS, Milne SB, Shibata N, et al. Regulated accumulation of desmosterol integrates macrophage lipid metabolism and inflammatory responses. Cell. 2012;151(1):138-52.
162. Huang SC, Everts B, Ivanova Y, O'Sullivan D, Nascimento M, Smith AM, et al. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. Nat Immunol. 2014;15(9):846-55.
163. Divakaruni AS, Hsieh WY, Minarrieta L, Duong TN, Kim KKO, Desousa BR, et al. Etomoxir Inhibits Macrophage Polarization by Disrupting CoA Homeostasis. Cell Metab. 2018;28(3):490-503.e7.
164. Mogilenko DA, Haas JT, L'Homme L, Fleury S, Quemener S, Levavasseur M, et al. Metabolic and Innate Immune Cues Merge into a Specific Inflammatory Response via the UPR. Cell. 2019;177(5):1201-16.e19.
165. Nomura M, Liu J, Rovira, II, Gonzalez-Hurtado E, Lee J, Wolfgang MJ, et al. Fatty acid oxidation in macrophage polarization. Nat Immunol. 2016;17(3):216-7.
166. Marcovecchio PM, Thomas GD, Mikulski Z, Ehinger E, Mueller KAL, Blatchley A, et al. Scavenger Receptor CD36 Directs Nonclassical Monocyte Patrolling Along the Endothelium During Early Atherogenesis. Arterioscler Thromb Vasc Biol. 2017;37(11):2043-52.
167. Febbraio M, Podrez EA, Smith JD, Hajjar DP, Hazen SL, Hoff HF, et al. Targeted disruption of the class B scavenger receptor CD36 protects against atherosclerotic lesion development in mice. J Clin Invest. 2000;105(8):1049-56.
168. Seimon TA, Nadolski MJ, Liao X, Magallon J, Nguyen M, Feric NT, et al. Atherogenic lipids and lipoproteins trigger CD36-TLR2-dependent apoptosis in macrophages undergoing endoplasmic reticulum stress. Cell Metab. 2010;12(5):467-82.
169. Kuchibhotla S, Vanegas D, Kennedy DJ, Guy E, Nimako G, Morton RE, et al. Absence of CD36 protects against atherosclerosis in ApoE knock-out mice with no additional protection provided by absence of scavenger receptor A I/II. Cardiovasc Res. 2008;78(1):185-96.
170. Sheedy FJ, Grebe A, Rayner KJ, Kalantari P, Ramkhelawon B, Carpenter SB, et al. CD36 coordinates NLRP3 inflammasome activation by facilitating intracellular nucleation of soluble ligands into particulate ligands in sterile inflammation. Nat Immunol. 2013;14(8):812-20.
171. Park YM, Febbraio M, Silverstein RL. CD36 modulates migration of mouse and human macrophages in response to oxidized LDL and may contribute to macrophage trapping in the arterial intima. J Clin Invest. 2009;119(1):136-45.

172. Gautier EL, Huby T, Saint-Charles F, Ouzilleau B, Pirault J, Deswaerte V, et al. Conventional dendritic cells at the crossroads between immunity and cholesterol homeostasis in atherosclerosis. Circulation. 2009;119(17):2367-75.
173. Birnberg T, Bar-On L, Sapoznikov A, Caton ML, Cervantes-Barragan L, Makia D, et al. Lack of conventional dendritic cells is compatible with normal development and T cell homeostasis, but causes myeloid proliferative syndrome. Immunity. 2008;29(6):986-97.
174. Paulson KE, Zhu SN, Chen M, Nurmohamed S, Jongstra-Bilen J, Cybulsky MI. Resident intimal dendritic cells accumulate lipid and contribute to the initiation of atherosclerosis. Circ Res. 2010;106(2):383-90.
175. Yun TJ, Lee JS, Machmach K, Shim D, Choi J, Wi YJ, et al. Indoleamine 2,3-Dioxygenase-Expressing Aortic Plasmacytoid Dendritic Cells Protect against Atherosclerosis by Induction of Regulatory T Cells. Cell Metab. 2016;23(5):852-66.
176. Macritchie N, Grassia G, Sabir SR, Maddaluno M, Welsh P, Sattar N, et al. Plasmacytoid dendritic cells play a key role in promoting atherosclerosis in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol. 2012;32(11):2569-79.
177. Doring Y, Manthey HD, Drechsler M, Lievens D, Megens RT, Soehnlein O, et al. Auto-antigenic protein-DNA complexes stimulate plasmacytoid dendritic cells to promote atherosclerosis. Circulation. 2012;125(13):1673-83.
178. Chen HJ, Tas SW, de Winther MPJ. Type-I interferons in atherosclerosis. J Exp Med. 2020;217(1):e20190459.
179. Daissormont IT, Christ A, Temmerman L, Sampedro Millares S, Seijkens T, Manca M, et al. Plasmacytoid dendritic cells protect against atherosclerosis by tuning T-cell proliferation and activity. Circ Res. 2011;109(12):1387-95.
180. Oh H, Mohler ER, 3rd, Tian A, Baumgart T, Diamond SL. Membrane cholesterol is a biomechanical regulator of neutrophil adhesion. Arterioscler Thromb Vasc Biol. 2009;29(9):1290-7.
181. Westerterp M, Fotakis P, Ouimet M, Bochem AE, Zhang H, Molusky MM, et al. Cholesterol Efflux Pathways Suppress Inflammasome Activation, NETosis, and Atherogenesis. Circulation. 2018;138(9):898-912.
182. Yvan-Charvet L, Ng LG. Granulopoiesis and Neutrophil Homeostasis: A Metabolic, Daily Balancing Act. Trends Immunol. 2019;40(7):598-612.
183. Yvan-Charvet L, Welch C, Pagler TA, Ranalletta M, Lamkanfi M, Han S, et al. Increased inflammatory gene expression in ABC transporter-deficient macrophages: free cholesterol accumulation, increased signaling via toll-like receptors, and neutrophil infiltration of atherosclerotic lesions. Circulation. 2008;118(18):1837-47.
184. Guasti L, Dentali F, Castiglioni L, Maroni L, Marino F, Squizzato A, et al. Neutrophils and clinical outcomes in patients with acute coronary syndromes and/or cardiac revascularisation. A systematic review on more than 34,000 subjects. Thromb Haemost. 2011;106(4):591-9.
185. Milligan G, Alvarez-Curto E, Hudson BD, Prihandoko R, Tobin AB. FFA4/GPR120: Pharmacology and Therapeutic Opportunities. Trends Pharmacol Sci. 2017;38(9):809-21.

186. Williams JW, Martel C, Potteaux S, Esaulova E, Ingersoll MA, Elvington A, et al. Limited Macrophage Positional Dynamics in Progressing or Regressing Murine Atherosclerotic Plaques-Brief Report. Arterioscler Thromb Vasc Biol. 2018;38(8):1702-10.
187. Ross S, Gerstein HC, Eikelboom J, Anand SS, Yusuf S, Pare G. Mendelian randomization analysis supports the causal role of dysglycaemia and diabetes in the risk of coronary artery disease. Eur Heart J. 2015;36(23):1454-62.
188. Ahmad OS, Morris JA, Mujammami M, Forgetta V, Leong A, Li R, et al. A Mendelian randomization study of the effect of type-2 diabetes on coronary heart disease. Nat Commun. 2015;6:7060.
189. Emami H, Singh P, MacNabb M, Vucic E, Lavender Z, Rudd JH, et al. Splenic metabolic activity predicts risk of future cardiovascular events: demonstration of a cardiosplenic axis in humans. JACC Cardiovasc Imaging. 2015;8(2):121-30.
190. Oburoglu L, Tardito S, Fritz V, de Barros SC, Merida P, Craveiro M, et al. Glucose and glutamine metabolism regulate human hematopoietic stem cell lineage specification. Cell Stem Cell. 2014;15(2):169-84.
191. Sarrazy V, Viaud M, Westerterp M, Ivanov S, Giorgetti-Peraldi S, Guinamard R, et al. Disruption of Glut1 in Hematopoietic Stem Cells Prevents Myelopoiesis and Enhanced Glucose Flux in Atheromatous Plaques of ApoE(−/−) Mice. Circ Res. 2016;118(7):1062-77.
192. Nagareddy PR, Murphy AJ, Stirzaker RA, Hu Y, Yu S, Miller RG, et al. Hyperglycemia promotes myelopoiesis and impairs the resolution of atherosclerosis. Cell Metab. 2013;17(5):695-708.
193. van der Valk FM, Kuijk C, Verweij SL, Stiekema LCA, Kaiser Y, Zeerleder S, et al. Increased haematopoietic activity in patients with atherosclerosis. Eur Heart J. 2017;38(6):425-32.
194. Freemerman AJ, Zhao L, Pingili AK, Teng B, Cozzo AJ, Fuller AM, et al. Myeloid Slc2a1-Deficient Murine Model Revealed Macrophage Activation and Metabolic Phenotype Are Fueled by GLUT1. J Immunol. 2019;202(4):1265-86.
195. Morioka S, Perry JSA, Raymond MH, Medina CB, Zhu Y, Zhao L, et al. Efferocytosis induces a novel SLC program to promote glucose uptake and lactate release. Nature. 2018;563(7733):714-8.
196. Fukuzumi M, Shinomiya H, Shimizu Y, Ohishi K, Utsumi S. Endotoxin-induced enhancement of glucose influx into murine peritoneal macrophages via GLUT1. Infect Immun. 1996;64(1):108-12.
197. Huang SC, Smith AM, Everts B, Colonna M, Pearce EL, Schilling JD, et al. Metabolic Reprogramming Mediated by the mTORC2-IRF4 Signaling Axis Is Essential for Macrophage Alternative Activation. Immunity. 2016;45(4):817-30.
198. Covarrubias AJ, Aksoylar HI, Yu J, Snyder NW, Worth AJ, Iyer SS, et al. Akt-mTORC1 signaling regulates Acly to integrate metabolic input to control of macrophage activation. Elife. 2016;5:e11612.
199. Namgaladze D, Zukunft S, Schnutgen F, Kurrle N, Fleming I, Fuhrmann D, et al. Polarization of Human Macrophages by Interleukin-4 Does Not Require ATP-Citrate Lyase. Front Immunol. 2018;9:2858.

200. Folco EJ, Sheikine Y, Rocha VZ, Christen T, Shvartz E, Sukhova GK, et al. Hypoxia but not inflammation augments glucose uptake in human macrophages: Implications for imaging atherosclerosis with ^{18}fluorine-labeled 2-deoxy-D-glucose positron emission tomography. J Am Coll Cardiol. 2011;58(6):603-14.
201. Sluimer JC, Gasc JM, van Wanroij JL, Kisters N, Groeneweg M, Sollewijn Gelpke MD, et al. Hypoxia, hypoxia-inducible transcription factor, and macrophages in human atherosclerotic plaques are correlated with intraplaque angiogenesis. J Am Coll Cardiol. 2008;51(13):1258-65.
202. Bjornheden T, Levin M, Evaldsson M, Wiklund O. Evidence of hypoxic areas within the arterial wall in vivo. Arterioscler Thromb Vasc Biol. 1999;19(4):870-6.
203. Vink A, Schoneveld AH, Lamers D, Houben AJ, van der Groep P, van Diest PJ, et al. HIF-1 alpha expression is associated with an atheromatous inflammatory plaque phenotype and upregulated in activated macrophages. Atherosclerosis. 2007;195(2):e69-75.
204. Parathath S, Mick SL, Feig JE, Joaquin V, Grauer L, Habiel DM, et al. Hypoxia is present in murine atherosclerotic plaques and has multiple adverse effects on macrophage lipid metabolism. Circ Res. 2011;109(10):1141-52.
205. Chaudhari SM, Sluimer JC, Koch M, Theelen TL, Manthey HD, Busch M, et al. Deficiency of HIF1alpha in Antigen-Presenting Cells Aggravates Atherosclerosis and Type 1 T-Helper Cell Responses in Mice. Arterioscler Thromb Vasc Biol. 2015;35(11):2316-25.
206. Aarup A, Pedersen TX, Junker N, Christoffersen C, Bartels ED, Madsen M, et al. Hypoxia-Inducible Factor-1alpha Expression in Macrophages Promotes Development of Atherosclerosis. Arterioscler Thromb Vasc Biol. 2016;36(9):1782-90.
207. Haschemi A, Kosma P, Gille L, Evans CR, Burant CF, Starkl P, et al. The sedoheptulose kinase CARKL directs macrophage polarization through control of glucose metabolism. Cell Metab. 2012;15(6):813-26.
208. Robbins CS, Hilgendorf I, Weber GF, Theurl I, Iwamoto Y, Figueiredo JL, et al. Local proliferation dominates lesional macrophage accumulation in atherosclerosis. Nat Med. 2013;19(9):1166-72.
209. Van den Bossche J, Baardman J, Otto NA, van der Velden S, Neele AE, van den Berg SM, et al. Mitochondrial Dysfunction Prevents Repolarization of Inflammatory Macrophages. Cell Rep. 2016;17(3):684-96.
210. Krawczyk CM, Holowka T, Sun J, Blagih J, Amiel E, DeBerardinis RJ, et al. Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. Blood. 2010;115(23):4742-9.
211. Everts B, Amiel E, van der Windt GJ, Freitas TC, Chott R, Yarasheski KE, et al. Commitment to glycolysis sustains survival of NO-producing inflammatory dendritic cells. Blood. 2012;120(7):1422-31.
212. Amiel E, Everts B, Fritz D, Beauchamp S, Ge B, Pearce EL, et al. Mechanistic target of rapamycin inhibition extends cellular lifespan in dendritic cells by preserving mitochondrial function. J Immunol. 2014;193(6):2821-30.
213. Thwe PM, Pelgrom LR, Cooper R, Beauchamp S, Reisz JA, D'Alessandro A, et al. Cell-Intrinsic Glycogen Metabolism Supports Early Glycolytic

Reprogramming Required for Dendritic Cell Immune Responses. Cell Metab. 2017;26(3):558-67.e5.

214. Lu H, Yao K, Huang D, Sun A, Zou Y, Qian J, et al. High glucose induces upregulation of scavenger receptors and promotes maturation of dendritic cells. Cardiovasc Diabetol. 2013;12:80.
215. Borregaard N, Herlin T. Energy metabolism of human neutrophils during phagocytosis. J Clin Invest. 1982;70(3):550-7.
216. Riffelmacher T, Clarke A, Richter FC, Stranks A, Pandey S, Danielli S, et al. Autophagy-Dependent Generation of Free Fatty Acids Is Critical for Normal Neutrophil Differentiation. Immunity. 2017;47(3):466-80.e5.
217. Rodriguez-Espinosa O, Rojas-Espinosa O, Moreno-Altamirano MM, Lopez-Villegas EO, Sanchez-Garcia FJ. Metabolic requirements for neutrophil extracellular traps formation. Immunology. 2015;145(2):213-24.
218. Jha AK, Huang SC, Sergushichev A, Lampropoulou V, Ivanova Y, Loginicheva E, et al. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. Immunity. 2015;42(3):419-30.
219. Liu PS, Wang H, Li X, Chao T, Teav T, Christen S, et al. alpha-ketoglutarate orchestrates macrophage activation through metabolic and epigenetic reprogramming. Nat Immunol. 2017;18(9):985-94.
220. Tavakoli S, Downs K, Short JD, Nguyen HN, Lai Y, Jerabek PA, et al. Characterization of Macrophage Polarization States Using Combined Measurement of 2-Deoxyglucose and Glutamine Accumulation: Implications for Imaging of Atherosclerosis. Arterioscler Thromb Vasc Biol. 2017;37(10):1840-8.
221. Sonoki T, Nagasaki A, Gotoh T, Takiguchi M, Takeya M, Matsuzaki H, et al. Coinduction of nitric-oxide synthase and arginase I in cultured rat peritoneal macrophages and rat tissues in vivo by lipopolysaccharide. J Biol Chem. 1997;272(6):3689-93.
222. Ren B, Van Kampen E, Van Berkel TJ, Cruickshank SM, Van Eck M. Hematopoietic arginase 1 deficiency results in decreased leukocytosis and increased foam cell formation but does not affect atherosclerosis. Atherosclerosis. 2017;256:35-46.
223. Kierszenbaum F, Wirth JJ, McCann PP, Sjoerdsma A. Impairment of macrophage function by inhibitors of ornithine decarboxylase activity. Infect Immun. 1987;55(10):2461-4.
224. Tarasenko TN, Singh LN, Chatterji-Len M, Zerfas PM, Cusmano-Ozog K, McGuire PJ. Kupffer cells modulate hepatic fatty acid oxidation during infection with PR8 influenza. Biochim Biophys Acta. 2015;1852(11):2391-401.
225. Yurdagul A, Jr., Subramanian M, Wang X, Crown SB, Ilkayeva OR, Darville L, et al. Macrophage Metabolism of Apoptotic Cell-Derived Arginine Promotes Continual Efferocytosis and Resolution of Injury. Cell Metab. 2020;31(3):518-33.e10.
226. Ponnuswamy P, Ostermeier E, Schrottle A, Chen J, Huang PL, Ertl G, et al. Oxidative stress and compartment of gene expression determine

proatherosclerotic effects of inducible nitric oxide synthase. Am J Pathol. 2009;174(6):2400-10.

227. Everts B, Amiel E, Huang SC, Smith AM, Chang CH, Lam WY, et al. TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKvarepsilon supports the anabolic demands of dendritic cell activation. Nat Immunol. 2014;15(4):323-32.
228. Guak H, Al Habyan S, Ma EH, Aldossary H, Al-Masri M, Won SY, et al. Glycolytic metabolism is essential for CCR7 oligomerization and dendritic cell migration. Nat Commun. 2018;9(1):2463.
229. Liu J, Zhang X, Chen K, Cheng Y, Liu S, Xia M, et al. CCR7 Chemokine Receptor-Inducible lnc-Dpf3 Restrains Dendritic Cell Migration by Inhibiting HIF-1alpha-Mediated Glycolysis. Immunity. 2019;50(3):600-15.e15.
230. Luchtefeld M, Grothusen C, Gagalick A, Jagavelu K, Schuett H, Tietge UJ, et al. Chemokine receptor 7 knockout attenuates atherosclerotic plaque development. Circulation. 2010;122(16):1621-8.
231. Potteaux S, Gautier EL, Hutchison SB, van Rooijen N, Rader DJ, Thomas MJ, et al. Suppressed monocyte recruitment drives macrophage removal from atherosclerotic plaques of Apoe$^{-/-}$ mice during disease regression. J Clin Invest. 2011;121(5):2025-36.
232. Wan W, Lionakis MS, Liu Q, Roffe E, Murphy PM. Genetic deletion of chemokine receptor Ccr7 exacerbates atherogenesis in ApoE-deficient mice. Cardiovasc Res. 2013;97(3):580-8.
233. Miller A, Nagy C, Knapp B, Laengle J, Ponweiser E, Groeger M, et al. Exploring Metabolic Configurations of Single Cells within Complex Tissue Microenvironments. Cell Metab. 2017;26(5):788-800.e6.

How to cite this article:
Gallerand A, Stunault MI, Merlin J, Guinamard RR, Yvan-Charvet L, Ivanov S. Myeloid Cell Diversity and Impact of Metabolic Cues during Atherosclerosis. Immunometabolism. 2020;2(4):e200028. https://doi.org/10.20900/immunometab20200028