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Lipids and lipoproteins in the interstitial tissue fluid regulate the formation of dysfunctional tissue-resident macrophages: Implications for atherogenic, tumorigenic, and obesogenic processes

Miriam Lee-Rueckert [a], Matti Jauhiainen [b], Petri T. Kovanen [a], Joan Carles Escolà-Gil [c,d,e,*]

[a] Wihuri Research Institute, Helsinki, Finland
[b] Minerva Foundation Institute for Medical Research, Biomedicum, Helsinki, Finland
[c] Institut de Recerca Sant Pau (IR SANT PAU), Barcelona, Spain
[d] Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Barcelona, Spain
[e] CIBER de Diabetes y Enfermedades Metabólicas Asociadas, CIBERDEM, Madrid, Spain

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ABSTRACT

An inflammatory and lipid-enriched tissue microenvironment is a common characteristic of the extracellular niches of affected tissues in atherosclerosis, cancer, and obesity. These respective interstitial environments appear to be induced by infiltration of plasma lipids and early local recruitment of monocyte-derived macrophages. In the tissue niches, the macrophages display remarkable phenotypic and functional plasticity and exert multifaceted roles in tissue homeostasis. Multiple local signaling events contribute to the phenotypic polarization of the tissue-resident macrophages into M1-like, M2-like, and multiple mixed subpopulations. This study aims to review and compare the roles of lipids and lipoproteins in shaping the inflammatory and lipid-enriched microenvironments of atherosclerotic arterial intima, malignant tumors, and obese adipose tissue, so generating dysfunctional macrophages. Circulating lipoprotein-bound lipids and albumin-bound fatty acids can cross the vascular endothelial barrier and infiltrate the interstitial fluids, resulting in variable levels of plasma-derived lipids, notably low-density lipoproteins (LDL), high-density lipoproteins (HDL), and locally generated small lipid-poor HDL species (preβ-HDL). Whilst LDL particles mainly supply liver-derived cholesterol to the cells of peripheral tissues, HDL particles can promote the reverse transfer of cellular cholesterol into the circulation and ultimately via the liver to the gut for its fecal excretion. Thus, the LDL/HDL ratio in the interstitial fluids can either promote or prevent cholesterol accumulation in the tissue-resident macrophages. Various types of peripheral cells modify interstitial LDL and HDL particles by oxidation, proteolysis, lipolysis, aggregation, or fusion, rendering them dysfunctional. By engulfing excessive amounts of extracellular lipids and modified LDL particles in such lipid-rich microenvironments, macrophages can become dysfunctional, a typical example being the atherosclerotic arterial intima. Similarly, tissue microenvironments characteristic of several malignant tumors and the obese adipose tissue are rich in triglyceride (TG)-rich lipoproteins and free fatty acids, inducing accumulation of TG and glycerophospholipids in the intracellular lipid droplets of macrophages. The lipid-loaded macrophages are currently considered novel markers for diagnosis and selective therapeutic targets not only in the arterial intima but also in malignant tumors and obese adipose tissue. Together, the available data identify potential roles of lipids and lipoproteins present in the interstitial fluids of the atherosclerotic arterial intima, malignant tumors, and obese adipose tissue in the generation of distinct lipid-loaded macrophage subpopulations and suggest their contributory roles in the development and progression of atherosclerosis, cancer, and obesity, the three major health concerns worldwide.

1. Introduction

Atherosclerosis, cancer, and obesity are chronic inflammatory diseases with major contributions to all causes of disabilities and human death worldwide, which notably share complex pathophysiological mechanisms and risk factors. Epidemiological and lipidomic studies

* Correspondence to: Institut de Recerca Sant Pau, C/ Sant Quintí 77, Barcelona 08041, Spain.
E-mail address: jescola@santpau.cat (J.C. Escolà-Gil).

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have disclosed versatile and deleterious associations between the plasma lipids and lipoproteins profile and the development of these diseases [1,2]. Cardiovascular disease and cancer often occur in the same individuals, in part due to concurrent risk factors such as overweight and obesity. A major mechanistic consideration is that obesity promotes adipose tissue (AT) inflammation, which is pathogenically linked to both cardiovascular disease and cancer [3,4]. Obesity has been clinically associated with the development of atherosclerosis, abdominal aortic aneurysm, heart failure, and certain malignant tumors such as gastric cancer, and a common link factor appears to be a variable status of chronic low-grade inflammation, which accelerates the onset or progression of atherosclerotic plaques [4] and tumors [5]. The increase in cancer risk with the more excess weight a person gains and the longer time a person is overweight was demonstrated in a large-scale population study in the UK [6]. This study also estimated that a 1 kg/m^2 population-wide increase in body mass index (BMI) would result in 3790 additional annual UK patients developing one of the ten cancer types found to be positively associated with BMI.

Macrophages are phagocytic cells of the innate immune system present in nearly all vertebrate tissues and organs, which, via diverse transcriptional features and functional capabilities, play central roles in maintaining tissue homeostasis and defense against infections [7]. They are versatile cells with a high degree of phenotypic and functional plasticity in different tissues and display multifaceted roles in diseases such as atherosclerosis, cancer, Alzheimer's disease, multiple sclerosis, and diabetes mellitus [8]. Thus, disturbances in tissue homeostasis that alter the local microenvironment can induce changes in the phenotype of tissue-resident macrophages to adapt these cells to variations in their niche. Due to their plasticity and multiple functions, macrophages can exert either detrimental or beneficial actions once becoming dysfunctional, e.g., by secreting key inflammatory mediators macrophages play critical roles in initiating and maintaining inflammation and its potential resolution, as well [9,10]. The net balance between the production of pro- and anti-inflammatory cytokines will ultimately determine the outcome of the pathogenic response in the target tissue [11]. Notably, macrophages can phagocytose neighboring cells alive, whether they are senescent cells or not, resulting in "cell death by phagocytosis", and they can also phagocytose apoptotic dying cells, which is generally conceived as "dead cell clearance", as classically occurs in the AT [12]. A complex interaction of specific antigens drives the phagocytosis process and a set of signaling pathways present both in the target cells to be phagocytosed and on the surface of macrophages, which ultimately determines whether a cell is phagocytosed by a phagocyte or not [13]. As a mechanistic example, a key negative regulator of macrophage activity is the cluster of differentiation (CD)47, a membrane-bound protein overexpressed by cancer cells [14], which operates as a "don't eat me" signal suppressing the phagocytic activity of macrophages. Moreover, in atherosclerotic lesions, the cellular stress in apoptotic cells fails to decrease their expression of CD47, and so reduces their engulfment by macrophages, which, again leads to the growth and ultimate instability of the lesions [15]. Whether advances in the current understanding of such fascinating phagocytic code could contribute to accelerating phagocytosis of cancer cells and hypertrophic adipocytes by macrophages constitutes a promising therapeutic option in the early treatment of both clinical disorders.

Dysfunctional macrophages have been described in the settings of atherosclerosis, cancer, obesity, other inflammatory diseases, and infections, hence representing a potential target for preventing and treating these diseases [16]. Defective macrophage responses promoting tissue damage are especially relevant if such imbalances occur in lipid-rich environments where macrophages are crucially involved in lipid uptake and turnover [17]. Endothelial dysfunction and increased vascular permeability characterize several metabolic disorders in which enhanced passage of macromolecules, such as plasma lipoproteins and even circulating cells, occurs towards interstitial fluids where tissue macrophages reside. Notably, macrophages possess specialized organelles, i.e., the lysosomes, where internalized lipids and lipoproteins are digested, and the lipid droplets composed of a hydrophobic core of neutral lipids, such as triglycerides (TG) and cholesteryl esters (CE), surrounded by an amphipathic phospholipids (PL) monolayer. Early studies described the lipid droplet as a passive intracellular cargo; however, further studies on lipid droplet biology have revealed their essential roles in regulating cellular homeostasis, including protection from reactive oxygen species and endoplasmic reticulum stress [18]. Macrophages also express scavenger receptors, which facilitate lipid uptake from different sources available in interstitial fluids, including fatty acids (FA), native and modified LDL particles, and TG-rich lipoproteins and their remnant particles. Upon storing excessive lipid loads in lipid droplets, macrophages become lipid-laden cells. Besides their capacity to buffer excess lipid cargos, lipid droplets can finely regulate a subsequent lipid release based on specific cellular requirements. Moreover, by sequestering toxic lipids within their neutral lipid core, the formation of lipid droplets can also prevent cellular lipotoxicity [19].

The main lipid classes stored in intracellular lipid droplets of macrophages and other cells are TG and CE, both types of lipid exerting relevant physiological and pathological functions. Excessive TG accumulation in the adipocytes of visceral AT leads to obesity and its associated metabolic syndrome [20], whilst disturbed cholesterol homeostasis that leads to macrophage CE accumulation in the arterial intima is the pathological basis of atherosclerosis. Moreover, cholesterol-lowering clinical interventions are also of benefit in the treatment of an increasing number of pathological disorders, such as neurodegenerative diseases, diabetes, obesity, as well as prostate cancer and other specific tumors [21]. TG are the major form of storage and transport of FA to peripheral cells from the intestine and liver in the core of chylomicrons and very low-density lipoproteins (VLDL), respectively. In normocholesterolemic states, most of the cellular cholesterol resides in the plasma membrane, where it modulates its fluidity and the intramembrane transport of many metabolites. The LDL particles-associated cholesterol is taken from the circulation into cells by the regulated LDL-receptor (LDL-R)-mediated pathway, while cellular cholesterol is released mainly to HDL particles by a process known as cellular cholesterol efflux, which occurs by different mechanisms of varied efficiencies [22,23].

The fact that macrophage numbers increase in the atherosclerotic lesion [24], many types of solid tumors [25,26], and the obese AT [27] strongly indicates a key role in the progression of these pathological disorders and it has fueled the design of novel macrophage-based therapies [28–30]. Nevertheless, to exert their pathophysiological functions, macrophages do not work in solitaire but must act in coordination with soluble components of the extracellular matrix (ECM) and via cell-to-cell interactions with a surrounding plethora of cells. Besides adipocytes and tumor cells, other non-macrophage cells cohabitate in tissues where macrophages reside, including smooth muscle cells (SMC), fibroblasts, dendritic cells, neutrophils, mesenchymal stem cells, T-cells, and mast cells. Such intricated cellular networks influence macrophage recruitment and stimulate phenotypic divergent switches associated to specific transcriptional programs. Furthermore, enzymes secreted to the ECM by macrophage neighboring cells can modify LDL and HDL particles mainly by inducing oxidation, proteolysis, and lipolysis. Both enzymatic and nonenzymatic modifications potentially occurring in the interstitial fluids can affect the respective pro- and anti-atherogenic effects of LDL and HDL on macrophages, i.e., by enhancing LDL capacity to induce cholesterol influx and reducing HDL capacity to induce cholesterol efflux [23,31].

Accumulation of lipids in abundant intracellular lipid droplets provides macrophages a bubbly or "foamy" appearance under the microscope, so they are named "foam cells." Although this term was first introduced in the atherosclerosis field to denote the typical cholesterol-loaded macrophages in atherosclerotic lesions, most lipid-loaded macrophages in other diseases display a similar foamy appearance. In the following sections, we approach how extracellular lipids can locally

induce lipid-loading of tissue-resident macrophages, thereby challenging their functions within the specific microenvironments of atherosclerotic lesions, malignant tumors, and the obese AT, contributing to disease progression. Paradoxically, although macrophage lipid accumulation induces pathogenic features driving inflammatory responses, it has been shown that under certain pathological conditions, transcriptional reprogramming can be initiated in the foam cells towards a macrophage anti-inflammatory phenotype [32].

2. Ontogeny of tissue-resident macrophage

Current advances in the knowledge of macrophage ontogeny are contributing to a better understanding of the complex functions of tissue-resident macrophages across organ development. Notably, data from pulse-labeling studies aimed at mapping the fate of developing macrophage cells in mouse embryos have provided evidence that significant macrophage populations are derived from embryonic progenitors and are capable of renewing independently of the input from bone marrow-derived hematopoietic stem cells [33,34]. These findings highlighted a dual origin of macrophages in adult tissues, challenging the traditional view suggesting that blood monocytes driven by the monocyte chemotactic protein 1 (MCP-1, also known as CCL2) were the major source of replenishing tissue macrophages [35]. However, the exact nature of the embryonic progenitors that give rise to adult tissue-resident macrophages is still under debate, and the mechanisms enabling macrophage adult population maintenance are not well-defined yet [36]. Furthermore, whether macrophages of distinct origins are functionally interchangeable or have unique roles is still a subject of active research.

Recent reports have indicated that hematopoietic stem cell-derived monocytes may render distinct macrophage populations in tissues and that a specific fraction of tissue-resident macrophages may depend on constant monocyte replenishment during adulthood. Despite the identification of many distinct macrophage phenotypes, it remains unclear if they represent functionally distinct conserved macrophage subsets or are determined by their ontogeny, or if their plasticity allows them to switch between functional states after a challenge such as an environmental trigger, immune activation, or tissue regeneration [36]. Single-cell RNA sequencing (scRNA-seq) across 17 murine tissues demonstrated that monocyte-derived macrophages distinctly contribute to 3 macrophage subsets with conserved life cycles based on common life cycle properties and core gene signatures. Moreover, monocytes contributed to the specific replacement of each identified murine macrophage population until a finite upper limit was reached, after which they did not outcompete the pre-existing resident macrophages [37]. These data revealed that specific tissue-resident macrophage subpopulations have a unique ontogeny relationship with blood monocytes, at least in the mouse.

Another study on the ontogeny/function relation of arterial macrophages found that in response to angiotensin-II inflammation, the increase in adventitial macrophages was driven by recruitment of bone marrow monocytes, while erythromyeloid progenitors-derived macrophages proliferated locally and provided a distinct transcriptional response linked to tissue regeneration [38]. The incomplete knowledge about the ontogeny of tumor-associated macrophages (TAMs) currently represents a challenge for cancer therapy. Although macrophages of different origins are known to accumulate within the tumor microenvironment (TME) in mouse cancer models, transcriptomic analysis highlighting functional profiles of resident vs. recruited TAMs has not been able, so far, to fully associate specific functional profiles with TAM origin across different tumor models [39]. Regarding the potential role of macrophage ontogeny on the function of the adipose tissue macrophages (ATMs), new genetic mouse models have provided novel insights by showing that yolk sac-derived macrophages promote postnatal expansion of adipocytes during early development and control adipocyte hypertrophy in obese mice, whilst bone marrow-derived monocytes/macrophages are primarily involved in AT inflammation, ectopic fat deposition, and insulin resistance [40]. Because identification of the two independent macrophage hematopoietic lineages was achieved only recently by applying emergent technologies, seminal functional studies performed in the last decade did not distinguish between macrophage lineages. Overall, further research on the tissue-specific functions of distinct macrophage subsets of different origins, among them the macrophage phagocytic capacity, set a promise to design targeted clinical intervention strategies for those inflammatory diseases where macrophages play a central role.

3. Polarization of tissue-resident macrophages induced by cytokines, growth factors, and environmental lipids

In various cell culture conditions, activated macrophages can be differentiated *in vitro* from human monocytes in response to specific cocktails of cytokines and growth factors into proinflammatory M1 and alternatively activated anti-inflammatory M2 phenotypes with distinct metabolic signatures [41]. M1-type macrophages rely mainly on glycolysis as the predominant energy supply, whereas M2-type macrophages are more dependent on FA β-oxidation and oxidative phosphorylation [42]. Although the M1/M2 classification has been a useful research tool, it is unlikely that it can account for the complexity of the transitional states that macrophages undergo during their activation. This is of most relevance in living tissues, where polarization may be finely tuned in response to a cocktail of cytokines and growth factors present in their microenvironments [43]. Thus, tissue-resident macrophages are simultaneously stimulated by a variety of signals and, accordingly, will be polarized into different subtypes [44]. In fact, application of high-dimensional approaches such as mass cytometry and scRNA-seq analysis to macrophages found across various normal or pathological human tissue dissected specimens has demonstrated their higher diversity and further revealed finer levels of heterogeneity [45].

Macrophage differentiation is under the influence of the macrophage-colony stimulating factor (M-CSF) and the granulocyte-macrophage colony-stimulating factor (GM-CSF), which are hemopoietic growth factors that regulate the recruitment and terminal differentiation of pluripotent cells in several tissues. M-CSF is constitutively expressed and ubiquitously produced by many cell types, while GM-CSF is mainly produced by activated leukocytes and synthesized in large amounts under various inflammatory conditions [46]. Most experimental studies on the effects of one of the two CSFs in cell proliferation and differentiation have attempted to facilitate the conversion of bone marrow or circulating monocytes into macrophage subtypes in cell culture conditions. *In vitro* models have shown that each CSF regulates the differentiation of mononuclear phagocytic cells into specific mature macrophage lineage populations by affecting their functional properties [47]. Overall, *in vitro* differentiation of macrophages with GM-CSF or M-CSF has been described to prime the cells toward proinflammatory (M1) or anti-inflammatory (M2) phenotype, respectively, although several overlapping features also exist [48,49]. The scavenger receptor CD36 is highly expressed in M2-like macrophages generated *in vitro* under the influence of M-CSF, which favors the formation of M2 foam cells when exposed to modified LDL [50]. The M-CSF-polarized M2-like macrophages also can accumulate cholesterol even when incubated with native, unmodified LDL [51].

It is worth denoting that several discrepancies in data derived from the vast number of *in vitro* cell culture studies may be related to the lack of uniform standards in differentiation protocols, e.g., the exposure to a single CSF or both CSFs simultaneously, the duration of exposure, and the composition of the culture media. In this context, adding serum to culture media in which human macrophages are differentiated can affect the results given the compositional lot-to-lot variation in commercial serum preparations [52]. Following this practical notion, we have shown that under macrophage serum-free culture conditions, the enhanced foam cell-forming potential of M-CSF-polarized human

macrophages does not require any additional serum-derived components [50]. Clearly, more well-defined cell culture conditions using time-varying variables covering the various stages along the long path from a monocyte to an activated macrophage are key issues to understand better the complex effects of the tissue microenvironment in which macrophage polarization occurs *in vivo*.

Although all macrophages share common immune signatures, reparative roles, and inflammatory responses to many stimuli, they also can express distinct tissue-specific developmental programs. Thus, the remarkable plasticity of human macrophages allows them to dynamically shift to various functional subtypes expressing mixed M1/M2 phenotype markers. This is exemplified by the characterization of various subpopulations of TAMs within the microenvironment of solid malignant tumors, which consist of pro-tumoral M2-like and a small fraction of anti-tumoral M1-like macrophages; however, a continuous spectrum leading to multiple discrete macrophage subtypes has been reported [53]. Thus, under variable inflammatory conditions occurring in their specific microenvironments macrophages can display a vast array of activation phenotypes beyond the M1/M2 range and can also produce phenotype shifts as a mechanism to maintain metabolic homeostasis, as it occurs during the development of malignant tumors and the onset of obesity [54]. Importantly, emerging studies in cancer utilizing scRNA-seq and spatial transcriptomics have expanded the classification of TAMs and, thus, opened a new era of macrophage-targeted cancer therapies [55]. Similarly, scRNA-seq analysis have advanced our knowledge revealing multiple macrophage signatures in atherosclerotic plaques and identifying novel phenotypes beyond the traditional M1/M2 paradigm [56].

Nuclear receptors, such as the ligand-activated transcription factors sterol regulatory element-binding proteins (SREBPs), liver X receptors (LXRs), and peroxisome-proliferator-activated receptors (PPARs) are key participants in macrophage polarization by controlling macrophage gene expression [57–60]. For instance, a recent report in human monocyte-derived macrophages generated under the influence of tumor-derived ascitic fluid showed that LXR activation impaired the acquisition of the transcriptional and functional properties of the M2 phenotype and skewed macrophages towards a pro-inflammatory gene signature [61]. In contrast, activation of SREBP1a, the predominant SREBP isoform in macrophages [62], by IL-4 stimulates *de novo* lipogenesis inducing macrophage polarization into the M2 phenotype [63]. Moreover, lipidomic analysis have demonstrated that signaling pathways of lipid metabolism involving glycerophospholipids, such as cardiolipins, and sphingolipids, such as ceramides, also in their oxidized forms, are key regulatory molecules in macrophage polarization and are able to shape the immune and inflammatory outcomes [64].

Various environmental lipids, including PL and their oxidized forms, can also function as signaling molecules that regulate macrophage polarization, thereby shaping immune and inflammatory responses. PL oxidation products accumulate in murine and human atherosclerotic lesions where they can induce lipid accumulation in macrophages by interaction with CD36 [65]. In this context, it was found that treatment of human monocyte-derived macrophages with oxidized 1-palmitoyl-2-arachidonoyl-sn-3-phosphorylcholine affected macrophage gene expression and function, resulting in development of a macrophage phenotype, distinct from M1/M2 conventional phenotypes, that involved the redox regulated transcription factor (Nrf2) [66]. A novel mechanism by which tumor cell apoptosis modulates macrophage polarization also identified phosphatidyl serine (PS) released from apoptotic cells as an inductor of M2-like macrophage polarization in tumor models *in vitro* and *in vivo* [67]. Thus, macrophage stimulation with PS liposomes reduced the expression of pro-inflammatory cytokines IL-1α, IL-1β, IL-12, IL-6, and tumor necrosis factor-alpha (TNF-α), enhanced the secretion of IL-10 and transforming growth factor-beta (TGF-β) and the expression of the M2 macrophage biomarker CD206, all conducive to the differentiation of macrophages toward the M2-like phenotype [67]. Also, administration of phosphatidyl ethanolamine to apoE$^{-/-}$ mice decreased the expression of inflammatory factors such as CCL2, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion protein-1 (VCAM-1), increased the aortic expression of SR-BI, a phenotypic marker of M2 macrophages, and promoted the transformation of macrophages into an anti-inflammatory M2 type [68]. Similarly, treatment with the ω3-polyunsaturated FA docosahexaenoic acid (DHA) resulted in upregulation of M2 macrophage markers through the p38 mitogen-activated protein kinase (MAPK) signaling pathway and increased the secretion of anti-inflammatory cytokines by U937-derived macrophages [69]. Oxidized-LDL particles (ox-LDL) which are present in the atherosclerotic intima, the TME, and the obese AT can also mechanistically induce M2-type macrophage polarization via cell signaling, metabolic reprogramming, epigenetic regulation, and intercellular regulation [70].

Overall, the relative abundance of different polarization types of macrophages in a given tissue may change dynamically via recruitment of polarized monocytes from the blood and/or through the effects of local factors on macrophages in the tissues [71]. Surprisingly, the mixed subpopulations of polarized macrophages present in atherosclerotic plaques, malignant tumors, and the obese AT can survive for long periods of time despite the toxic environments present in these body compartments, where also hypoxia and a low interstitial fluid pH prevail [32,72,73].

4. Lipids in interstitial fluids and lipid loading of tissue-resident macrophages

The phenotype diversity observed in transcriptional profiling studies of tissue-resident macrophages is most likely controlled by key transcription factors and epigenetic regulators induced in response to tissue-specific environments [74]. In tissues, macrophages interact with lipid components of the interstitial fluids, which are typically composed of water, electrolytes, and plasma-derived components, including LDL and HDL. Macrophages also crosstalk with their neighboring cells by expressing membrane receptors, releasing secretory products, and eliminating extracellular debris by phagocytic clearance of dying cells. The presence of lipid-loaded macrophages that have been developed under multiple metabolic, infectious, and autoimmune conditions across different tissues has led to the conclusion that their pathogenetic formation involves disease-specific mechanisms [75]. Foam cells can be formed when tissue-resident macrophages avidly internalize lipids and lipoproteins available in high concentrations in interstitial fluids. A golden example is the macrophage uptake of native and modified LDL particles in the arterial intima and the skin, typically inducing the formation of cholesterol-laden foam cells, characteristic of atherosclerotic lesions and dermal xanthomas.

Albumin is the main non-esterified or free FA (FFA) binding protein in extracellular fluids, forming albumin-FA complexes in plasma. Yet, most of the plasma lipids are bound to the various types of lipoprotein particles, which mainly carry cholesterol and TG. To infiltrate peripheral tissues, lipids must cross the semipermeable barrier of the vascular endothelium that regulates the solute composition of interstitial fluids by an ultrafiltration process of blood components through the endothelial cell barrier [76]. Small molecules may cross this inner vascular layer according to concentration gradients. In contrast, the larger molecules and cells can only cross via transcytosis processes mediated by vesicles and receptors or through impaired endothelial junctions, leading to an enhanced endothelium permeability. Overall, the lipid movement from the circulation across the endothelial barrier is known to be coordinated by the capillary lipoprotein lipase (LPL) that is attached to the luminal surface of endothelial cells, and it involves endothelial cell binding proteins, lipid receptors, and nonreceptor passive mechanisms [77]. After their dissociation from albumin or their lipolytic release from the core of TG-carrying lipoproteins by the LPL, the FFA most likely transverse the endothelium by transporter-facilitated mechanisms, including the peripheral membrane FA binding protein [78]. Although

traditionally, the transit of lipoproteins through the endothelium was considered to occur by passive filtration across a dysfunctional endothelial barrier, this dogma was challenged by the identification of several rate-limiting cellular receptors facilitating transcytosis. Thus, plasma lipoproteins, once crossing the endothelial glycocalyx at the apical endothelial side, are exposed to specific endothelial receptors that transfer them into the interstitial fluids via caveolae-mediated transcytosis to be finally released from the basolateral side of the endothelial cells. The transcytosis routes of the cholesterol-carrying lipoproteins LDL and HDL across the endothelial barrier appear to be tissue-specific and involve the scavenger receptor class B member 1 (SR-B1), activin like kinase 1, and caveolin-1 for LDL as well as SR-B1, the transmembrane lipid receptors ATP-binding cassette transporter (ABC)A1 and G1, and endothelial lipase for HDL [79,80]. Currently, the mechanisms and molecular regulation facilitating the endothelial transcytosis of lipoproteins remain poorly understood, partly due to technical limitations in observing the process *in vivo*.

The movement of plasma lipoproteins toward the interstitial fluid is driven by their respective plasma concentrations and sizes, which also dictates the efficiency of TG lipolysis by LPL at the luminal surface of the endothelial cells. The ultimate lipoprotein composition of the interstitial fluid is also influenced by lipoprotein remodeling factors, namely the cholesteryl ester transfer protein (CETP) and the phospholipid transfer protein (PLTP). Notably, the human lymph-to-plasma ratio of CETP was found to be lower than that of PLTP [81], which might relate to the fact that plasma CETP is bound to HDL and other larger lipoproteins that do not cross the endothelium readily [82]. Thus, the relative activity levels of lipid transfer proteins in human interstitial fluids locally generate an abundant local subpopulation of small discoidal lipid-poor preβ-HDL particles [81].

The normal endothelial inter-junctional space (3–6 nm), which represents a steric hindrance for the passive filtration of macromolecules such as LDL (18–25 nm) and HDL (7–14 nm) through the endothelial junctions, can be challenged under pathological conditions that induce a leaky dysfunctional endothelium as typically surrounding an atherosclerotic lesion [83], a solid tumor [84], and the obese AT [85]. Under these conditions, the augmented vascular permeability significantly increases the size of the endothelial cell-cell junctions and so promotes the passage of large blood solutes like the albumin-FA complexes and lipoproteins and even facilitates the transendothelial migration of various immune cells.

Tissue-resident macrophages exposed to lipid-rich interstitial fluids, as in conditions of increased vascular permeability, can be easily converted into lipid-loaded macrophages by the enhanced lipid uptake via distinct cellular pathways, including passive or receptor-facilitated diffusion and active mechanisms mediated by membrane transporters. Detection of lipid droplets in macrophages with the use of the fluorescent dye BODIPY gave rise to the general term “lipid-associated macrophages” (LAMs) to describe macrophages with lipid-handling capacity identified in diseased tissues with lipid metabolic derangements such as the obese adipose AT and certain tumors, which can be generated by recruitment and differentiation from circulating monocytes or by tissue-resident macrophage repolarization [86]. Endocytic, pinocytic, and phagocytic processes can also increase the macrophage lipid load. Via fluid-phase pinocytosis and expression of CD36 and scavenger receptor class A1 (SRA1), macrophages can internalize FFA as well as native and modified LDL becoming lipid-loaded cells [87–89]. CD36 plays a key role in the formation of lipid-loaded macrophages and it was found that M2 activation with interleukin (IL)-4 upregulates CD36 expression, whereas M1 activation with lipopolysaccharide (LPS) and interferon-γ resulted in downregulation of the receptor [90], which is in line with the general notion that M2-polarized macrophages have a greater lipid-scavenging potential. In this regard, it is essential to note that the specific composition and availability of lipids in the interstitial fluid vary widely in different tissues, influencing the type of lipids ingested and stored by macrophages. Since lysosomes play an important role in the digestion of all sorts of macromolecule loads, macrophage lipid accumulation may also occur by lysosomal dysfunction induced by defects in the catabolic machinery or by excess lipid substrates impairing their efficient breakdown.

Given the fact that cholesterol cannot be degraded by mammalian cells, any excess is either stored in lipid droplets or exported via cholesterol efflux pathways. Cholesterol-laden macrophages are formed in the arterial intima of atherosclerotic blood vessels upon their exposure to high LDL concentrations in the intimal fluid. Excessive internalized LDL-derived cholesterol is accumulated as CE in lipid droplets of the foamy macrophages. Similarly, cholesterol-loaded macrophage foam cells formed in the skin lead to the development of cutaneous xanthomas. Lipid loading of TAM subpopulations from malignant tumors and of ATMs from the obese AT has been also described but, in contrast to the vast information on the lipid droplet composition of the macrophage foam cells typical of atherosclerosis, the type of accumulated lipids, their potential origins, and the pathophysiological role of the lipid cargo in TAM and ATM populations are uncleared [73]. Overall, the scarce data about lipid-loaded TAMs and lipid-loaded ATMs indicate that they accumulate primarily TG with variable levels of CE and glycerophospholipids, as it will be described in the following sections. Given the critical role of cholesterol-loaded macrophage foam cells in the development of atherosclerotic lesions, the reverse cholesterol transport (RCT) component that initiates in macrophages (macrophage-specific RCT, M-RCT) is considered a valuable tool in experimental studies to prevent or induce regression of atherosclerosis. Because of the remarkable differences in lipid composition of lipid-loaded TAMs and lipid-loaded ATMs compared to the atherosclerosis-associated cholesterol-loaded macrophage foam cells, evaluation of the M-RCT rate in experimental studies on obesity and cancer appears irrelevant.

Finally, the changes in lipid homeostasis that lipid-loaded macrophages undergo, by modifying the rates of lipogenesis, FA β-oxidation, lipolysis, and lipid uptake and transport, can also influence macrophage polarization. In general, because M2 macrophages primarily utilize FA oxidation and oxidative phosphorylation for energy, while M1 macrophages tend to rely more on glycolysis, variation in lipid homeostasis affecting these metabolic processes will affect their polarization. Thus, whether macrophages are loaded with cholesterol or other lipids, formation of the foam cells leads to a non-static state of polarization by expressing endogenous signaling molecules like nuclear receptors and transcription factors [91,92]. However, it is worth mentioning that conditions prevalent in the ECM of inflamed tissues, such as hypoxia and acidity, and the distinct mixtures of oxidized lipids and secreted cytokines and growth factors will further interact with macrophage foam cells in these body compartments and can stimulate a dynamic phenotype reprograming.

5. Cholesterol-laden macrophages in the atherosclerotic arterial intima

The key risk factors of atherosclerosis are of environmental and/or genetic origins. In patients affected with atherosclerotic cardiovascular disease of non-genetic origin, the elevation of circulating LDL can occur at any age but usually constitutes a threat in adult life. In these patients, the disease is associated with sustained high blood pressure, inappropriate dietary patterns, and smoking, among other environmental factors [93]. In contrast, in subjects affected by the rare hereditary disease homozygous familial hypercholesterolemia (HoFH), extremely high levels of LDL appear in early life, leading to extensive cholesterol deposition not only in the arterial intima but also in the skin, typically forming dermal lesions or xanthomas [94]. HoFH is caused by LDL-R deficiency, as will be described in the next section. Concerning the atherosclerotic lesion development in the intima it has been long known that, independently of its origin, the generation of cholesterol-loaded macrophage foam cells is strictly linked to the high levels of

apolipoprotein (apo)B-containing lipoproteins in the matrix of the intima layer of blood vessels, a process in which LDL serve as the primary source of cholesterol [95]. The lesion is aggravated by subsequent pathways of programmed cell death, including apoptosis, autophagy, necroptosis, and pyroptosis that the lesional foam cells undergo, thereby building the necrotic core of advanced atherosclerotic plaques [96]. Another relevant characteristic of human atherosclerotic plaques are tissue hypoxia and acidification [97,98], which are triggered when intimal macrophages become dysfunctional.

Although there is also experimental evidence on the atherogenicity of TG-rich lipoproteins, e.g., VLDL, intermediate density lipoproteins (IDL), and chylomicrons and their remnants [99], there is not yet any consensus about targeting elevated levels of these lipoproteins to prevent cardiovascular diseases. In contrast, because tissue-resident macrophages constitute a significant population of intimal cells that avidly take up native and modified forms of LDL via fluid-phase pinocytosis or scavenger receptor-mediated pathways, an indissoluble direct link between LDL, macrophages, and atherogenesis has been established [100]. Indeed, a broad cohort study analyzing pooled data from 4 prospective cohort studies in the US clearly showed that maintaining an optimal level of LDL-cholesterol throughout young adulthood and middle age can minimize the lifetime risk for atherosclerotic cardiovascular disease [101]. Despite vascular SMC, endothelial cells, and dendritic cells can also take up cholesterol derived from intimal LDL particles and thereby may also become cholesterol-loaded foam cells, macrophages are considered the primary source of foam cells in the atherosclerotic lesions [95]. Also, activated macrophages have a very high energy demand and they preferentially use glycolysis for ATP synthesis even under normoxic conditions, resulting in enhanced local generation and secretion of lactate and protons, which locally decrease the extracellular pH [72].

In contrast to other tissues in which the concentration of LDL particles is only one-tenth of that in the circulation, in the arterial intima their concentration equals that in the circulation [102]. LDL can enter the arterial wall by transcytosis or by passive movement across an injured or dysfunctional endothelial barrier [79]. Interestingly, it was recently hypothesized that most of the accumulating LDL reaches the intima not from the lumen but from the adventitia and media arterial layers. This directional transmural cholesterol flux (TCF) suggests that excess LDL diffusing from the avascular (i.e., devoid of blood and lymph vessels) media's SMC, is cleared by the endothelium through its apical membrane. According to this novel view, a plaque would be formed when the cholesterol clearance rate lags behind its rate of arrival by the TCF mechanism [103]. Indeed, the uniquely high concentration of LDL in the intimal fluid prevails due to the lack of lymphatic capillaries in the intimal space, which is a closed space separated from the medial layer of the arterial wall by a largely impermeable internal elastic lamina [104]. Efficient lymphatic LDL drainage is prevented because the intima lacks lymphatic capillaries, and they appear only in very advanced lesions. Moreover, unlike HDL, the movement of LDL particles in the intima is restricted because of LDL selective binding to arterial proteoglycans (PG) locally secreted by SMC, which impedes their intimal clearance [105]. LDL interaction with a dense ECM network rich in PG, collagen, and elastin initiates LDL retention in the intima and significantly favors the formation of various forms of modified LDL particles via oxidative, proteolytic, and lipolytic processes, which enhances their proatherogenic effects. PG are the major LDL-binding component of the arterial wall, and LDL retention occurs on their chondroitin sulfate/dermatan sulfate glycosaminoglycan chains [106]. SMC are major producers of PG in the intima, and it has been demonstrated that changes in the glycosaminoglycan chains induced by exposure of SMC to high levels of albumin-bound FA increased LDL binding *in vitro* [107], suggesting that modification of proteoglycan synthesis and structure may be a prime target to prevent LDL binding and entrapment in the vessel wall and, thus, preventing atherosclerosis development and progression [108].

The non-regulated uptake of modified LDL particles by scavenger receptors plays a major role in foam cell formation. It was estimated that 75–90 % uptake of LDL modified by chemical acetylation or oxidation is mediated by the macrophage CD36 and SRA1 receptors *in vitro* [89]. Hypoxia *per se* has been found to increase macrophage-mediated LDL oxidation by increasing the expression of 15-lipoxygenase-2, which suggests that the hypoxic microenvironment of atherosclerotic lesions may play a specific role in the generation of ox-LDL species of higher atherogenicity [109]. Ox-LDL is rapidly recognized and phagocytosed by CD36 on the surface of macrophages leading to lipid loading and triggers the signaling cascade of the inflammatory response, process that has been inhibited by phosphatidylethanolamine (PE) supplementation to macrophages *in vitro* [110]. Ox-LDL promote foam cell formation by a mechanism primarily mediated by CD36 through the production of reactive oxygen species, which then triggers activation of a member of the NOD-like receptor (NLR) family, the canonical NLRP3 inflammasome component, and ultimately resulting in the maturation and secretion of IL-1β, a key inflammatory cytokine that contributes to foam cell formation [111]. More recently it was found that NLRP11, may provide a novel checkpoint control for NLRP3 inflammasome assembly [112], however, the NLRP3 inflammasome response in humans is still incompletely understood.

Also, native LDL can be taken up by macrophages via receptor-independent fluid-phase pinocytosis and inducing foam cell formation [113]. Despite both GM-CSF and M-CSF have been found in circulation and within atherosclerotic plaques where they can induce the generation of M1 or M2 macrophage phenotypes [114], immunostaining of human coronary arteries has shown that macrophages with similar antigen expression to M2-like macrophages were predominant within atherosclerotic lesions, whereas macrophages expressing markers of M1-like phenotype predominated in areas devoid of disease [51]. Independently of the foam cell-forming mechanism, the intracellular LDL-derived CE are hydrolyzed in lysosomes, and then cholesterol is re-esterified by the macrophage enzyme acyl CoA: cholesterol O-acyltransferase 1 (ACAT1) in the cytoplasmic compartment forming the microscopically visible CE droplets typical of foam cells, the hallmarks of atherosclerotic lesions [115]. Importantly, the low pH and oxygen deprivation characteristic of the intimal fluid of deep areas of atherosclerotic lesions can potentially modify macrophage functionality by affecting their lipid cargo. Thus, *in vitro* exposure of human macrophage foam cells to a low pH mimicking the acidic conditions prevailing in the atherosclerotic intima lowers their intracellular pH with a simultaneous decrease in the cholesterol-esterifying activity of ACAT1 [116]. Such pH sensitivity of ACAT1 may lead to intracellular accumulation of potentially toxic levels of FC, a contributing factor to macrophage foam cell death. Hypoxia was also found to affect lipid metabolism in macrophages by increasing the FA pool available for the biosynthesis of TG and CE. Thus, exposure of human macrophages to hypoxia for 24 h enhanced the formation of TG-containing cytosolic lipid droplets, which was attributable to increased TG biosynthesis, reduced FA β-oxidation, and increased expression of the adipocyte differentiation-related protein [117].

Macrophage cholesterol efflux stimulated by HDL can significantly compensate for the excessive influx of LDL-derived cholesterol and becomes a critical process to clear cholesterol from the arterial intima [118]. Cholesterol efflux occurs only in the form of free cholesterol (FC), and it depends on the type of extracellular cholesterol acceptors available and the efficiency of the cholesterol efflux mechanisms operating in the donor cell. Cholesterol efflux includes a passive bidirectional aqueous diffusion process, which SR-BI can facilitate, and active unidirectional pathways mediated by ABCA1 and ABCG1. Aqueous diffusion is the primary cholesterol efflux pathway in native macrophages, and it can account for up to~80 % of the total efflux in non-loaded mouse peritoneal macrophages [119]. In contrast, upon cholesterol loading, macrophages respond by inducing gene and protein expression of ABCA1 and ABCG1, which increases the efficiency of FC removal and can potentially attenuate the atherosclerotic burden by accelerating the M-RCT rate [120]. Nascent apoA-I-containing preβ-HDL particles are the

most efficient cholesterol acceptors and specific ligands of ABCA1, whereas the mature lipid-rich spherical α-HDL particles (HDL_2 and HDL_3) are the main physiological acceptors of cholesterol released by ABCG1 [120]. Macrophage synthesis of apoE also stimulates cholesterol efflux from cholesterol-loaded macrophages, via ABCA1-dependent mechanism [121]. The preβ-HDL particles travel via the lymphatics to the circulating blood where they it can be further modified to become α-HDL particles, which are ultimately removed from the circulation by the hepatic SR-B1 receptors [122]. ABCA1 can also facilitate PL efflux to lipid-free apoA-I [123,124]. More recently, modern omics technologies have provided deeper insights into the diverse lipid and protein compositions of HDL and preβ-migrating HDL particles, revealing that any disease-specific alterations are induced via changes in the proteome and lipidome of HDL, i.e., changes of the various molecular components present in the HDL particles [125,126]. Overall, these studies have revealed a high degree of particle speciation and diverse molecular signatures affecting the cholesterol efflux capacity of HDL [127–132]. In addition to the fact that the effects of the specific HDL-associated proteins and HDL subspecies on cholesterol efflux are still incompletely understood, the data collectively reveal that quantifying total plasma HDL abundance alone does not account for the protective or harmful effects of the various circulating HDL subspecies on disease risk [133].

Studies *in vitro* have demonstrated that an acidic pH, like such present in the intimal fluid that surrounds macrophage foam cells in areas of advanced atherosclerotic lesions, can disturb the ability of the foam cells to release cholesterol, particularly affecting the function of the ABCA1 transporter [134]. Moreover, HDL particles can lose their capacity to induce cholesterol efflux upon being exposed to extracellular enzymatic processes such as proteolysis, oxidation, and lipolysis promoted by activated cells present in the inflamed intima [135]. In addition, in advanced atherosclerosis, excess accumulation of extracellular cholesterol will crystalize to a solid state, forming abundant aggregates in advanced atherosclerotic plaques, which contributes to their core expansion, aggravates their inflammatory state [136], and can affect the endothelial barrier function thereby increasing vascular permeability to circulating lipoproteins [137]. The physiological HDL-mediated efflux cannot remove cholesterol crystals and, accordingly, by expanding the size of the necrotic core of the atherosclerotic plaque and thinning of the cap of the plaque, they promote the likelihood of its rupture that triggers local thrombus formation and thereby may precipitate an acute coronary syndrome [138]. An early study reported that administration of the cyclic oligosaccharide 2-hydroxypropyl-β-cyclodextrin to $apoE^{-/-}$ mice fed with a cholesterol-rich diet reduced the size of atherosclerotic plaques and their cholesterol crystal load [139]. Since using cyclodextrins in humans is approved as a solubilizing agent for lipophilic drug delivery, these drugs might be of potential pharmacological value in treating human atherosclerosis. However, to the best of our knowledge, further studies on this matter have not been published so far.

Interestingly, besides the critical foam cell-forming activity of LDL, these particles can also act as acceptors of FC released by the ABCG1-mediated pathway, albeit with much less efficiency relative to HDL [140,141]. Moreover, LDL can act as a reservoir of plasma cholesterol *in vitro* and *ex vivo* [141]. The observation that FFA accumulate in LDL when albumin is low or absent suggests a compensatory role of LDL acting as a transitory sink of bioactive molecules in the vascular compartment [142]. After reaching the circulation, both LDL and HDL will partially deliver their cholesterol cargo to the liver. Ultimately, hepatic cholesterol, as FC or after its conversion to bile acids, is secreted into the bile and intestine, completing the hepatobiliary RCT route to feces. Generation of intestinal HDL and systemic effects of the gut microbiota have revealed a direct link between the gut and the cholesterol cargo of peripheral macrophages [143]. A better understanding of the insight mechanisms guiding the direction of cholesterol fluxes from macrophage foam cells to the gut in vivo could ultimately contribute to applications for drug development, particularly in conditions of hypercholesterolemia and enhanced atherosclerosis. The recent finding that d-lactate, a minor gut metabolite, is capable of transforming M2-like into M1-like TAM and thereby remodeling the TME immunosuppressive property amplifies and further expands the significance of the gut microbiota to other pathological disorders, such as cancer [144]. The flux of cholesterol inward or outward intimal macrophages promoted by LDL and HDL and the liver-dependent route of macrophage-derived cholesterol into the intestine are detailed in Fig. 1.

The pathway facilitated by plasma CETP via LDL, rather than the direct HDL-CE uptake by SR-BI, is considered the major mechanism involved in the hepatic uptake of plasma CE (Fig. 1**, bottom**). Notably, LDL and LDL-R appear to act in concert to facilitate a CETP-independent M-RCT in which LDL mediate the transfer of FC from macrophages to feces [141]. A key modulator of the hepatic LDL-R function is the proprotein convertase subtilisin/kexin type 9 (PCSK9) secreted predominantly by the liver, which increases circulating LDL levels by directing hepatic LDL receptors into lysosomes for degradation. In humans, gain-of-function mutations in this gene yielded elevations in LDL-C levels and, conversely, individuals with loss-of-function in PCSK9 were protected from atherosclerosis [145]. In line with this notion, treatment with PCSK9 antibodies was found to enhance the M-RCT rate by upregulating the LDL-R and facilitating the transfer of LDL-derived cholesterol to feces, but only in heterozygous LDL-R-deficient mice expressing human apoB100, and not in mice with normal LDL-R activity [146]. Such an LDL-mediated reverse FC transport pathway may be initiated in peripheral cells, such as the subcutaneous dermal macrophage foam cells embedded in an interstitial fluid, where LDL can flow more freely and the cholesterol esterification rate is very low (see next section). In contrast, to exert such M-RCT contribution from the foam cells in the human intima, LDL particles should exit the intimal fluid and undergo backward diffusion to the circulation, which, as mentioned above, seems rather unlikely. Thus, LDL particles, once entering the arterial intima, instead of draining into the lymph to regulate RCT, will remain trapped in the dense ECM and, when modified, will be taken up by the macrophage foam cells. Accordingly, LDL would stimulate macrophage cholesterol inflow rather than efflux.

Finally, it is worth mentioning that the attributed association of the immune response driven by cholesterol-loaded foam cells with chronic inflammatory responses in the artery wall has been challenged by recent findings indicating that cholesterol-laden macrophages do not directly drive inflammation and instead could elicit anti-inflammatory macrophage phenotypes [147,148]. Indeed, transcriptome analysis of isolated intimal foamy and nonfoamy macrophages from murine atherosclerotic aortas of $Ldlr^{-/-}$ and $Apoe^{-/-}$ mice showed that foamy macrophages expressed few inflammatory genes, while nonfoamy macrophages formed the major population expressing IL-1β and many other inflammatory transcripts [149]. This report is in line with other studies suggesting that in terms of the local atheroinflammatory component of atherogenesis, macrophage foam cell formation may be considered an anti-atherogenic event [50,150,151]. Yet, understanding of the ultimate role of foamy macrophage formation on the inflammatory response in atherosclerotic plaques is still controversial and remains incomplete. It is plausible that the final response in a given tissue may depend on the cocktail of bioactive molecules present in the respective microenvironment and the net balance among macrophage phenotypes with differing functions. Oxidized PL are considered critical modulators of inflammation and treatment with 2 types of oxidized phosphatidylcholines (PC) induced unique alterations in the macrophage lipidome of nonfoamy RAW264.7 macrophages amplifying their pro-inflammatory lipidomic signature [152]. Also, in mouse models of advanced atherosclerosis the C-type natriuretic peptide predominantly accumulated in macrophages, but significantly decreased in advanced plaques, promoted an anti-inflammatory macrophage phenotype, and reduced their conversion into foam cells [153]. Thus, the dogma-overriding novel hypothesis according to which the macrophage foam cells are less inflammatory than nonfoamy macrophages necessitates further clarification.

6. Cholesterol-laden dermal macrophages in cutaneous xanthomas

Xanthomas are small yellowish papules or nodules on the skin, typically formed on the joints, especially the knees and elbows, that occur due to the accumulation of lipid-laden macrophages under the skin's surface [94]. These skin lesions can occur in different types of acquired dyslipidemias. Still, they are commonly present in patients affected by the genetic disease HoFH in which the xanthoma severity and rate of progression are closely related to the development of atherosclerotic plaques in the arterial intima. In contrast to the vast number of studies on the lipid composition of atherosclerotic plaques, the specific lipid composition of a xanthoma has been poorly investigated and it appears to depend on the dyslipidemia and xanthoma types. An early study demonstrated that in the case of HoFH, the predominant xanthoma lipid is cholesterol, mostly as CE [154].

The skin consists of an outer layer of stratified epithelium, the epidermis, the dermis, and a subcutaneous layer called the hypodermis. The hypodermis is the deepest skin layer that lies closest to the muscle and mainly contains fat, which insulates the body from low temperatures and helps to absorb shock, thereby preventing damage to the internal organs. Other components include collagen-rich connective tissue and a network of blood vessels and nerves. The epidermis is populated by Langerhans cells, the dermis contains tissue-resident dermal macrophages, mast cells, and dendritic cells, and the hypodermis contains macrophages and T cells [155].

Because the arterial intima and the skin are the only two sites in the body where cholesterol-loaded macrophage foam cells are formed in response to high plasma levels of LDL-cholesterol, the pathogenetic mechanisms for the deposition of cholesterol involved in the development of xanthomas and early atherosclerotic lesions may be alike. Once it was demonstrated that quantification of cholesterol in the stratum

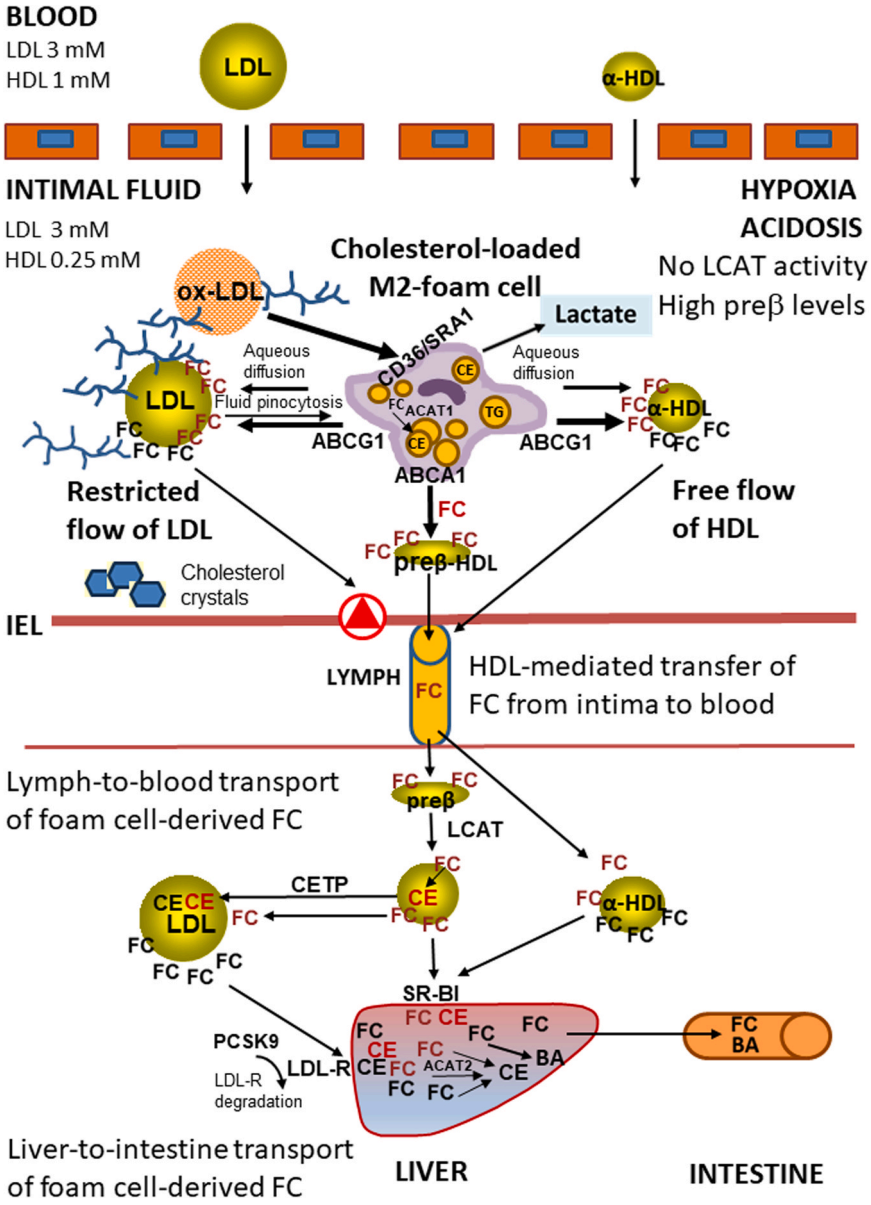

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Fig. 1. Lipoprotein-dependent cholesterol influx and efflux from cholesterol-laden macrophage foam cells in the arterial intima The balance of cholesterol in tissue-resident macrophages depends on the efficiency of the mechanisms governing LDL-depending influx and HDL-depending efflux of cholesterol. **TOP:** LDL and HDL cross the endothelium and filter into the arterial intimal fluid where macrophages reside. Because of the relatively small fenestrae in an intact IEL, the flux of LDL into the media is limited. Thus, unlike the interstitial fluids of the skin and other peripheral tissues, LDL particles accumulate in the intima, and their concentrations in the intimal fluid equal those in circulation. LDL intimal flow is also restricted due to LDL electrostatic binding to vascular PG of the ECM, causing the entrapped LDL to coalesce into large lipid-protein aggregates favoring LDL modifications, which generates modified LDL particles with proinflammatory properties, such as ox-LDL. Also, hypercholesterolemia disrupts the organization of the cell-to-cell endothelial junctions and opens the endothelial junctions, so increasing LDL entrance and deposition in the arterial intima. Modified LDL particles generated in the intima become ligands of macrophage scavenger receptors CD36 and SRA1, promoting the formation of CE-containing lipid droplets. High LDL concentrations in the intima also favor macrophage non-selective uptake of native LDL by fluid-phase micropinocytosis. High levels of macrophage-derived lactate are present in the atherosclerotic intima, which decreases the pH of the intimal fluid. In response to cholesterol loading, the expression of the cholesterol efflux transporters ABCA1 and ABCG1 increases and enhances the macrophage ability to efflux cholesterol to extracellular acceptors, mainly to HDL and, to a lesser extent, to LDL. Because of the absence of LCAT activity, the interstitial fluids are enriched in preβ-HDL particles, preferred ligands of ABCA1. A minor fraction of "free" LDL particles can act as an acceptor of FC from the foam cells, but it is unlikely that they can ultimately exit the intima. Cholesterol-laden macrophage foam cells eventually undergo apoptosis and death, the excess of extracellular cholesterol will crystalize to a solid state, and cholesterol crystals will contribute to inflammation and core expansion of the atherosclerotic plaque. **BOTTOM:** The lymphatic pathway to return LDL to the blood via lymph is impeded by the IEL that separates the arterial tunica intima from the tunica media and must abolish the contribution of LDL to the reverse movement of macrophage FC to circulation. In contrast, HDL particles carrying macrophage foam cell-derived FC, particularly preβ-HDL particles can readily flow across the IEL and are transferred via lymph from the intimal fluid to blood, thereby initiating the anti-atherosclerotic M-RCT route. After reaching the blood compartment, the plasma enzyme LCAT esterifies FC in the nascent preβ-HDL resulting in their remodeling into mature α-HDL particles, which contains a core of CE with a monolayer surface composed of apoA-I, other apolipoproteins, and PL. HDL-derived cholesterol is taken up selectively by the hepatic SR-BI receptor without internalization of the HDL particle. HDL-CE are also partly transferred to LDL by CETP, which along this pathway contributes to delivering cholesterol to the liver and, ultimately, into the intestinal lumen. LDL also serves as a transient storage pool of cell-derived FC until its elimination from blood by the hepatic LDL-R. PCSK9 released by the liver to the bloodstream promotes the breakdown of LDL-R on the surface of hepatocytes. To avoid toxicity, the excess of FC reaching the liver is re-esterified into CE by the hepatic enzyme ACAT2. Finally, both macrophage-derived and non-macrophage-derived cholesterol molecules flowing through the total body RCT are transferred in subsequent steps of the reverse pathway to the intestine as FC or after its conversion into BA. ***Abbreviations***: intimal elastic lamina (IEL), extracellular matrix (ECM), high-density lipoprotein (HDL), low-density lipoprotein (LDL), oxidized-LDL (ox-LDL), cluster of differentiation 36 (CD36), scavenger receptor class A1 SRA1, cholesteryl esters (CE), free cholesterol (FC), ATP-binding cassette transporter (ABC), lecithin:cholesterol acyltransferase (LCAT), macrophage-specific reverse cholesterol transport (M-RCT), scavenger receptor class B type I (SR-BI), cholesteryl ester transfer protein (CETP), LDL receptor (LDL-R), proprotein convertase subtilisin/kexin type 9 (PCSK9), acyl-coenzyme A:cholesterol acyltransferase (ACAT), bile acids (BA), triglycerides (TG).

corneum (the outermost layer of the epidermis) correlates with the epidermal cholesterol content, early application of this simple assay allowed to disclose a direct correlation existing between skin total cholesterol and the carotid intima-media thickness (CIMT) [156]. Over time, more reliable, non-invasive, and accurate methods for detecting skin cholesterol have been developed, and the direct association of skin cholesterol content with an increased CIMT has been proven consistent in numerous studies [157–160]. The overall results show that cholesterol changes in the skin mirror those in the arterial intima and that skin cholesterol content is an independent marker of practical value in identifying individuals with subclinical asymptomatic atherosclerosis.

The interstitial fluid of peripheral compartments like the skin contains about 10 % of the plasma lipoproteins and proteins that have crossed the endothelial layer of tissues. Under physiological conditions, both LDL and HDL can cross the normal intact endothelium of the skin by transcytosis. In contrast to the restricted movement of LDL in the intimal fluid, both LDL and HDL move freely within the interstitial fluid of the skin and interact with the population of dermal macrophages. It is plausible that the LDL-binding strength of different structural components of the skin, such as decorin, is weaker as compared with the vascular ECM [161]. Seminal studies in peripheral lymph have estimated that the extravascular pool of apoA-I in healthy men may be as large as the plasma pool (about 4 g) and that for every LDL in a human interstitial fluid, there are, on average, 50 or more HDL particles, whereas in the presence of a normal, non-leaky endothelium the larger size particles, i.e. chylomicrons and VLDL, are virtually absent [81]. Since aqueous diffusion is the main cholesterol efflux mechanism in cholesterol-normal (non-loaded) macrophages, whereas the ABC transporters contribute to a small extent, FC molecules will diffuse from the plasma membrane of dermal macrophages until colliding cholesterol acceptors in the interstitial fluid, mainly HDL and to a lesser degree LDL. Due to the higher affinity of LDL for FC, part of the FC pool present in HDL must be transferred to LDL via diffusion through the aqueous phase of the interstitial fluid. Because of the absence of LCAT activity in interstitial fluids, after efflux to lipoproteins, FC molecules released from macrophages will remain unesterified. A fraction of LDL and HDL, which become enriched in FC within the cutaneous interstitial fluid, will return to the blood via the lymphatic route. LDL acts as a transient storage pool of cell-derived FC until its elimination from the blood by the hepatic LDL-R. After reaching the blood compartment, LCAT-mediated esterification of FC remodels preβ-HDL into α-HDL particles, and part of the CE is transferred from α-HDL to LDL by CETP, thereby increasing the LDL contribution to the overall delivery of circulating cholesterol to the liver. Given the free flow of FC-enriched LDL from interstitial fluids via lymph to plasma, as well as the LDL role as a reservoir of FC in plasma, LDL and the LDL-R are capable of contributing significantly to the reverse transport of extrahepatic FC to the liver [141].

Although a low-efficiency cholesterol efflux mechanism, i.e., passive diffusion, predominates in dermal macrophages under normocholesterolemic conditions, being the skin the larger organ of the body, M-RCT from the skin must have a significant contribution to the total body-RCT. Hypercholesterolemia, by weakening the endothelial junctions and increasing vascular permeability by different mechanisms, favors LDL transcytosis, as shown in various animal models [162–165]. In the skin, the sole increase of endothelial permeability of dorsal skin sites by histamine in normocholesterolemic rodents has increased *in vivo* the transendothelial passage of plasma LDL and HDL to the cutaneous interstitial fluid of the affected sites [166,167]. Therefore, the ultimate effect of hypercholesterolemia on dermal macrophage foam cell formation will depend on the compensatory transendothelial passage of functional HDL into the interstitial fluid, which will determine the net macrophage cholesterol balance towards accumulation or clearance of skin cholesterol. Of note, proteolytic degradation and functional inactivation of preβ-HDL by chymase, a chymotrypsin-like serine protease derived from activated mast cells, has been demonstrated in mouse models *in vivo* [168]. The highest mast cell concentration in the human body is found in the skin; yet, although the mast cells are not activated in normal skin, in inflamed skin sites such as psoriatic lesions, they become stimulated and release various proteases, among them the chymase, such as occurs also in inflamed atherosclerotic lesions [169,170].

The phenotype of macrophages populating xanthomas has been poorly investigated. A case report of the histological findings in a 46-year-old woman affected by esophageal xanthoma described the immunohistochemistry of macrophage lineage markers expression,

which revealed that the foam cells observed in the lesion consisted of M2 macrophages and agreed with the higher capacity of this phenotype to generate foam cells [171]. HDL particles in FH patients display qualitative abnormalities, including compositional changes and reduced capacity to promote macrophage cholesterol efflux, contributing to an increased risk for premature cardiovascular events [172,173]. Strong LDL elevation and dysfunctional HDL particles induce accelerated formation of intracellular CE-containing lipid droplets, generating abundant macrophage foam cells in the arterial intima of the affected patients. An enhanced macrophage expression of ABC cholesterol efflux transporters is insufficient to prevent the accumulation of foam cell clusters also in the dermis, and abundant cutaneous xanthomas are frequent in severe conditions of FH, such as HoFH. Of note, lipid analysis of eruptive xanthomas observed in insulin-dependent hyperlipidemic patients has shown that the FA pattern of TG in xanthomas and circulating chylomicrons were similar, suggesting that hyperchylomicronemia also significantly contributed to the xanthoma lipids in these patients [174]. The molecular defect associated with these early clinical observations is unknown and deserves further investigation.

Xanthomas can also develop without hypercholesterolemia, further indicating that the ultimate cholesterol content in the skin will depend on the relative efficiency of macrophage cholesterol influx and efflux processes. Several HDL deficiency disorders significantly compromise macrophage cholesterol efflux and so impair the M-RCT rate, which has been related to the development of xanthomatosis, among them the rare genetic deficiency in apoA-I, the main HDL apolipoprotein. HDL deficiency in the absence of hyperlipidemia has been studied in rodents, as in the apoE/apoA-I double deficient mouse model, which was characterized by severe alterations of skin morphology associated with xanthoma formation, aortic and coronary atherosclerosis, and by local and systemic inflammation [175]. In humans, apoA-I deficiency is a rare monogenic metabolic disorder resulting in undetectable apoA-I levels and HDL-cholesterol lower than 20 mg/dL. Familial HDL deficiencies are associated with variable susceptibility to premature coronary heart disease, but the mechanism underlying this association is still incompletely understood. The few described HDL deficiency syndromes are originated from decreased or absent production of HDL particles, HDL hypercatabolism as in ABCA1 functional deficiency of Tangier disease (TD), or enzymatic defects such as LCAT deficiency or fish-eye disease (FED), among other causes. All these rare conditions share the same metabolic failure, i.e., compromised cholesterol efflux caused by HDL deficiency; however, the formation of xanthomas has not been systematically reported in all of them. Intriguingly, xanthomatosis has been observed early in the familial deficiency of apoA-l and apoC-lll, and in the HDL deficiency with planar xanthomas, but not in other HDL-deficient syndromes, as was reported in an early study [176].

A clinical study in Turkish kindred affected by the genetic HDL deficiency with xanthomas characterized by a genetic defect in the apoA-I gene proposed that, in the absence of functional apoA-I, the fraction of cellular cholesterol is released to an unstable population of circulating apoA-IV/LCAT particles and are rapidly transferred as CE into the VLDL/IDL density fraction. This factor, together with an impaired removal of remnant particles and the rapid conversion of these particles to LDL could explain the increased LDL levels present in this genetic disorder and its potential relation with xanthoma formation [177]. Taken together, the lack of HDL or impairment of HDL function in some of these disorders may compromise the M-RCT rate from the skin, resulting in peripheral accumulation of cholesterol and the development of cutaneous xanthomas. Partly because of the low prevalence of these genetic disorders, the mechanism involved in generating xanthomas and their full impact on atherosclerosis risk have not been fully assessed. Whether LDL particles may contribute to compensating for the compromised M-RCT rate under the above conditions is unknown.

In summary, the origin of lipid deposition in macrophage xanthomas is multifactorial, including the plasma lipoprotein profile, e.g., high LDL and/or low levels of HDL increase endothelial permeability and local inflammation. Notably, the formation of dermal cholesterol-loaded macrophage foam cells can be either caused by extreme hypercholesterolemia (typical of HoFH) or apoA-I deficiency (Table 1). Detection of ox-LDL in xanthomas and other dermatological disorders, as in psoriatic lesions [178], suggests that extravasated native LDL can be oxidatively modified by resident and infiltrating cells also in xanthoma lesions, where ox-LDL contributes to foam cells recruitment [179,180]. An early study found that LDL density substances extracted *ex vivo* from experimental rabbit xanthoma tissue were more electronegative than plasma native LDL and contained lipid-protein complexes that showed physiochemical properties of ox-LDL [181]. It is also feasible that circulating ox-LDL particles generated in the blood enter the peripheral interstitial fluids, where they can stimulate the formation of cholesterol-loaded macrophage foam cells such as in atherosclerotic lesions and xanthomas [182]. Of interest is a recent study on the murine aortic arch, which compared changes in LDL entry and retention before and during atherosclerosis development and reported that LDL retention is the overall limiting factor for LDL accumulation [183]. Whether this finding translates directly to the human setting remains to be disclosed. However, the extremely high capacity for LDL retention of the intima compared to the skin would favor the hypothesis that ox-LDL is generated in the intima favoring development of atherosclerotic lesions, whereas ox-LDL already in circulation is rather extravasated into the skin where these particles are capable to stimulate macrophage foam cells and xanthoma formation. This preliminary speculation awaits to be experimentally tested.

A comparison of the efficiency in macrophage cholesterol deposition between the arterial intima atheroma and the dermal xanthoma is open for speculation. Regarding the skin, the best possible example is the HoFH, which indicates that extremely (e.g. 7-fold) high LDL-cholesterol levels are needed to generate xanthomas. The retention of LDL particles (apoB-binding to extracellular PG) in the skin might be less strong than in the intima, unfortunately, to our best knowledge there is no available data characterizing the negative charge density of the skin PG. There might be also protective mechanisms toward xanthoma formation even in patients with otherwise high LDL-cholesterol levels (not since birth). Among them, the initial HDL-mediated RCT capacity appears to be not so efficient in the arterial intima exposed to the same blood HDL-cholesterol level as in the skin. Indeed, it has been long known that several extracellular HDL modifications can potentially occur in the atherosclerotic intimal fluid where activated cells secrete multiple enzymes capable of impairing the HDL cholesterol efflux capacity by oxidative, lipolytic, or proteolytic damage [135]. Of note, modified HDL particles have been detected in the human atherosclerotic intima. In comparison to plasma HDL, lesion-derived HDL contained 6- to 8-fold higher levels of 3-nitrotyrosine and 3-chlorotyrosine, likely produced by the myeloperoxidase enzyme present in human atheroma [184,185]. In comparison to the intima, the lymphatics are more active in the skin and, therefore, the passage dynamics of lipoproteins through the interstitial fluid is fast, so reducing the time for further local modifications. Finally, the atherosclerosis-sensitive segments of the coronary tree consist of medium-sized arteries, whereas in the skin the circulatory system feeding the local cells consists of capillaries, i.e., vessels that do not develop atherosclerosis. Instead, the lack of capillaries available for the return of the extravasated LDL and HDL particles appears to be a decisive proatherogenic or anti-atherogenic factor among even hypercholesterolemic subjects, except for HoFH. Therefore, the presence/absence of capillaries must be also considered when attempting to explain the different foam cell-forming responses of the coronary intima and the skin to elevated LDL levels.

7. Lipid-loaded macrophages in the tumor microenvironment

For uncontrolled growth and proliferation, cancer cells require specific stimulation and support from the surrounding elements within the tissue they originate, including stromal cells and non-cellular ECM

Table 1
Responses of skin-resident macrophages to cholesterol accumulation in conditions of normocholesterolemia (Normo), homozygous familial hypercholesterolemia (HoFH), and apoA-I deficiency (ApoA-I$^{-/-}$).

Condition	Circulating levels in blood			Local levels in dermal interstitial fluid			Cholesterol efflux mechanism			Cholesterol loading magnitude
	LDL-C mM	HDL-C mM	LCAT nmol/h/ml	LDL-C mM	HDL-C mM	LCAT nmol/h/ml	Aqueous diffusion	ABCA1	ABCG1	
Normo	3.0	1	30	0.3	0.2	0	+ +	+	+	-
HoFH	30	0.85	25	3.0	0.17	0	+	+ +	+ +	+ ++
ApoA-I$^{-/-}$	3.0	0	30	0.3	0	0	+	+ + no HDL	+ +	+ +

In the skin, macrophages are primarily located in the dermis layer. To prevent cholesterol overloading, dermal macrophages must release cholesterol to extracellular cholesterol acceptors, mainly to HDL. The interstitial fluid of peripheral compartments, like the skin, contains a fraction (about 10 %) of LDL particles, HDL particles, and plasma proteins that have crossed the endothelial layer. The absence of LCAT activity enriches the dermal interstitial fluid in preβ-HDL particles, which are efficient acceptors of cholesterol via the ABCA1 transporter. Also, α-HDL particles in the dermal interstitial fluid promote cellular cholesterol efflux via passive diffusion and ABCG1 pathway and can initiate M-RCT from tissue-resident macrophages. In tissues where the free flow of LDL occurs, as in the skin, LDL could moderately act as a cholesterol acceptor via passive aqueous diffusion and ABCG1 pathways, thereby transferring FC from interstitial fluids via lymph to plasma and contributing to M-RCT. In conditions of normocholesterolemia, cholesterol efflux from dermal macrophages occurs mainly by aqueous diffusion, whereas the contribution of ABC-mediated efflux is minor. Albumin, PL-containing liposomes, HDL, and LDL can act as acceptors of cholesterol by passive diffusion. High circulating levels of LDL, e.g., in the rare genetic condition of HoFH, induce accelerated LDL-derived cholesterol influx and formation of intracellular CE-containing lipid droplets in dermal macrophages generating cholesterol-laden foam cells. Macrophages respond to cholesterol accumulation by overexpressing ABCA1 and ABCG1 transporters, which promote cholesterol efflux with higher efficiency. ABCA1 is the major efflux transporter in foam cells releasing FC to the interstitial fluid enriched in preβ-HDL particles, whilst ABCG1 acts releasing FC to both α-HDL and LDL. In severe conditions of FH macrophage cholesterol influx exceeds efflux and accumulation of foam cell clusters in the dermis forms cutaneous xanthomas. Individuals affected by several HDL deficiency syndromes, e.g., the rare genetic condition of apoA-I deficiency, have null or extremely low HDL levels which compromise macrophage cholesterol efflux, and abundant dermal cholesterol-laden foam cells are formed. Notably, apoA-I deficiency generates cutaneous xanthomas, even in the presence of normal levels of LDL in plasma. ***Abbreviations***: lecithin: cholesterol acyltransferase (LCAT), ATP-binding cassette transporter (ABC), macrophage reverse cholesterol transport (M-RCT), free cholesterol (FC), phospholipids (PL), cholesteryl ester (CE), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C)

components comprising the TME. The TME of malignant tumors contains neoplastic cells that maintain intricate interactions with their surrounding stromal cells, fibroblasts, endothelial cells, and blood-derived cells, including macrophages, T cells, granulocytes, and mast cells [186,187]. Lipid-loaded macrophages are also present in the TME of several types of solid malignant tumors, where they interact with cancer cells to support tumor growth and progression or may exert immunosuppressive activities [188]. Due to different tumor etiology and nutritional status of cancer patients, conflicting results have been reported on the lipid and lipoprotein profile of cancer patients, which, as mentioned above, can influence the formation of lipid-loaded cells in tissues. Yet, high circulating LDL levels have been positively correlated with the development of certain malignant tumors e.g., it has been reported that every 10 mg/dL increase in plasma cholesterol increases the risk of recurrence of prostate cancer by 9 % [189].

TAM populations are found in the stromal compartment of virtually all types of solid tumors, where they respond to different stimuli by releasing a distinct repertoire of growth factors, cytokines, chemokines, and enzymes, which play critical roles at all stages of tumor progression [190]. It has been estimated that TAMs constitute-50 % of tumor mass and increased TAM numbers are related to the malignancy degree and poor prognosis of many kinds of tumors, such as in lung cancer, breast cancer, and lymphomas [191,192]. Cancer cells, such as in prostate and hepatocellular carcinomas, can produce CCL2, which promotes macrophage recruitment and increases expression of M2 markers *in vitro* [193]. In addition, distinct metabolic features of the TME such as hypoxia, nutrient deprivation, lactic acidosis, and accumulation of metabolic waste, occur in spatiotemporal fashions and contribute to create multiple coexisting TAM phenotypes [194,195]. Particularly, in desmoplastic rigid tumors, the nutrient availability within the TME is affected by a dense surrounding ECM which creates a challenge for the local vasculature to deliver oxygen and metabolites [196]. Also, a rapidly growing tumor causes high glucose consumption in the TME to meet the large energy needs of cancer cells, which tends to deplete the exogenous glucose levels. Solid hypoxic tumor cells oxidize glucose through glycolysis, and the end product of this pathway is fermented into lactate, which accumulates in the TME, thereby decreasing the pH of the tumor interstitial fluid [197].

Tumor cells can reprogram their energy metabolism to promote rapid cell growth and proliferation depending on the concentrations of external nutrients and different stress conditions. Similarly, TAMs undergo versatile metabolic reprogramming in the TME to optimize the available energy and metabolites to meet their biosynthetic needs. Lipid uptake mediated by CD36 and the preferred use of FA β-oxidation instead of glycolysis for energy were important in TAM lipid accumulation and their differentiation towards the M2-like phenotype [198]. In line with this finding, it was shown in a gastric cancer tumor-bearing mouse model that uptake of tumor-derived lipids or exogenous lipids by TAM resulted in lipid accumulation and polarization towards M2-like profiling. The lipid-loaded TAMs exhibited decreased phagocytic potency and enhanced anti-tumoral immunity [199]. Notably, by creating an acidic environment, tumor cells can upregulate key genes of lipid metabolism in neighboring TAMs, promoting lipid accumulation and inducing them to gain a tumor cell-supportive M2 phenotype that ultimately promotes cancer progression [200]. Although further studies are needed to fully understand the mechanisms involved and the signaling pathways associated with such reciprocal interactions, the knowledge of the metabolic changes and associated phenotypes of TAMs favoring the growth of malignant tumors has remarkably increased in recent years. Moreover, the growing evidence that many types of human tumors are enriched in lipid droplet-containing TAMs has stimulated further studies in the field of cancer research aimed at understanding the identity and impact of lipids present in the TME on specific lipid-loaded TAM subpopulations and the tumor lifecycle and prognosis [188,201]. Lipid loading is not restricted to macrophages in the TME since high FA uptake and accumulation of neutral lipids in lipid droplets also occur in cancer cells in response to hypoxia, which can improve cell survival within certain limits [202].

The fact that most TAMs have the pro-tumoral M2-phenotype might also relate to a potential influence of M-CSF on macrophage polarization via a complex cytokine-based connection within the TME [203,204]. M-CSF has been reported to be overexpressed and secreted by different types of human cancer cells [205,206]. Also, tumor cells secrete or induce the secretion of IL-4, IL-10, and IL-13, which prime immune cells and induce TAM polarization into the M2 phenotype [207,208]. Hypoxia and high lactate concentrations may be key drivers of recruitment and polarization of TAMs [209]. A differential effect of lactate harming the metabolism of anti-tumoral TAMs and promoting the suppressive

capacity and metabolic activity of the pro-tumoral TAM subtype was shown in a model of mouse lung carcinoma [210]. It was found that the G protein-coupled receptor 132 (Gpr132) functions as a key macrophage sensor of the rising lactate in the acidic tumor milieu, and a positive association was demonstrated between the increase in Gpr132 levels and M2-TAM infiltration and metastasis in breast cancer models in mice [211]. Notably, the receptor for lactate GPR81 and a lactate-stimulated signaling pathway hold by extracellular signal-regulated kinase (ERK)/signal transducer and activator of transcription 3 (STAT3), have emerged as critical regulators of tumor growth and metastasis in lung and breast cancers, respectively [212,213]. Tumor-derived lactate was also found to induce M2 macrophage polarization by activating the ERK/STAT3 signaling pathway [213] and, accordingly, lactate has been used as a signaling molecule to induce M2-like macrophages [214]. When secreted by human mesenchymal stromal cells, lactate has induced differentiation of monocytes into M2 macrophages in a dose-dependent manner [215]. Moreover, lactate released into the hypoxic TME as a by-product of glycolysis in cancer cells upregulates hypoxia-inducible factor 1-α (HIF-1α) expression in TAMs also leading to M2-like polarization and sustaining TAM pro-tumoral functions [216]. Conversion of M1- to M2-phenotype in the hypoxic and hypoglycemic TME further promotes cancer progression. Notably, several studies have shown that tumor hypoxia *per se* shapes and induces specific tumorigenic macrophage phenotypes that serve tumor malignancy by promoting immune evasion, angiogenesis, tumor cell survival, and metastatic dissemination [217]. All these findings indicate that lactic acidosis in the TME is essential to generate and maintain the M2-TAM phenotype with pro-tumoral properties. In addition, ischemia and hypoxia in tumor tissue could also lead to the accumulation of apoptotic cells. Since PS released from apoptotic tumor cells can induce macrophage polarization toward the immunosuppressive M2-like TAM phenotype [67], these findings extend our understanding of how the multiple interactions between TAMs and their microenvironment modify their pro-tumoral effect.

In tumors developed in tissues surrounded by obese AT, like the breast, cancer cells and adipocytes become obliged partners, increasing tumor size and mortality [218]. *In vitro* studies have revealed that breast cancer cells can stimulate lipolysis in mature adipocytes and accumulate adipocyte-derived FA, which, again, enhanced the cancer cell to proliferate and migrate [219]. Besides providing cellular metabolic substrates to cancer cells as an energy source, such an increase in the FFA pool of the TME also becomes available for CD36-mediated uptake by the TAM population, thus becoming lipid-loaded TAMs. An enhanced FFA pool in the TME could also result from (*i*) increased direct passage of plasma albumin-bound FA, (*ii*) FFA released from nearby adipocytes, and (*iii*) enhanced indirect and direct pathways of transcytosis of VLDL and LDL particles across a leaky endothelium [79]. During this latter event, FFA can be released by local actions of LPL and secreted phospholipase A2. Increased LPL expression and activity have been reported in several types of malignant tumors [201]. In accordance with this report, high amounts of FFA were detected in tumor interstitial fluids from rodent models, as well as acyl-carnitines, ceramides, and esterified cholesterol [220]. The FFA composition in the interstitial fluid of the TME plays a role in this matter. Indeed, an unsaturated FA-enriched environment affects the maturation and polarization of myeloid cells, and thus, oleate (but not stearate) treatment alone polarized macrophages into an M2-like TAM phenotype with a robust immunosuppressive capacity, which may support metastasis by tumor escape from immune surveillance [221]. Moreover, in *in vitro* polarization experiments, sodium oleate treatment induced the accumulation of lipid droplets only in tumor-infiltrating TAMs but not in the benign tissue of colorectal cancer patients [221].

Compared with normal cells, such as hepatocytes, tumor cells have exhibited a unique ability to transfer their lipids to macrophages and drive M2 pro-tumoral polarization via CD36 activity. Thus, in a clinical model of liver metastasis, the metastasis-associated macrophages had higher CD36 expression, contained more lipid droplets, and could engulf tumor cell-derived long-chain FA, which were carried in extracellular vesicles [222]. The referred study demonstrated CD36-mediated cooperative crosstalk linking cancer cells and TAM phenotype towards tumor growth and dissemination to other body compartments and indicates that targeting the tumor-macrophage metabolic interface via CD36 inhibition may be an effective treatment strategy against liver metastasis. Another distinct type of conserved lipid-associated TAMs (known also as LAMs) has been detected in the lungs of mice bearing metastatic mammary tumors employing the neutral lipid dye LipidTox staining [223]. Further evidence supporting a pro-tumoral role of CD36 was the finding that TAM across several human and murine tumor types expressed elevated levels of CD36, accumulated lipids, and used FA β-oxidation instead of glycolysis for energy, moreover, inhibiting FA uptake by blocking CD36 or knocking down Cd36 in TAMs blocked their conversion into lipid-loaded TAMs and abolished their protumor functions *in vitro* and *in vivo* [198]. Overall, the available data indicate that CD36 plays a key role inducing pro-tumoral TAM phenotypes in the TME. Given that the CD36 is a major player in immunosuppression driven by deregulated lipid metabolism in cancer cells, clinical trials using the CD36 inhibitors represent promising novel pharmacological approach in cancer therapeutics [224]. Interestingly, more than 30000 nucleotide variants were found in the human *CD36* gene which impact their function on fat intake and obesity, FA processing, HDL metabolism and other metabolic syndromes [225,226]. Nevertheless, the uptake of exogenous lipids by TAMs may also involve SRA1 and other lipoprotein receptors. A study in patients with prostatic adenocarcinoma showed that expression of the broad-specificity type macrophage class A scavenger receptor with collagenous structure (MARCO) and BODIPY staining were positively correlated in a TAM subpopulation associated with tumor progression. Mechanistically, cancer cell-derived IL-1β enhances MARCO expression in macrophages, and reciprocally, cancer cell migration was promoted by CCL6 released by lipid-loaded TAMs [227]. The fact that SRA1 is an essential marker of M2-like TAMs [228] suggests that this TAM phenotype has, indeed, increased the potential capacity to scavenge lipids from the TME through a broad set of scavenger receptors. Interestingly, immunohistochemical experiments in mouse melanoma models have revealed a high prevalence of ox-LDL in tumor interstitial fluids, which can be taken up by tumor-infiltrating lymphocytes and is also facilitated by increased expression of CD36 [220]. The origin of ox-LDL in the TME may be related to the prevailing hypoxic microenvironment in tumors and in atherosclerotic plaques [109]. Ox-LDL also induces polarization toward the M2 phenotype on bone marrow-derived macrophages via interaction with macrophage CD36 and the platelet-activating factor receptor [229]. Moreover, several studies have established that ox-LDL may be an independent mitogenic factor and may promote metastasis [230].

Scarce studies have reported the lipid composition of lipid-loaded TAMs. Besides TAM's capacity to scavenge lipids from the TME, cancer cells can also promote lipid biosynthesis in TAMs, favoring TAM foam cell formation. Two models of human tumor cells associated with high TAM infiltration demonstrated that under the influence of tumor cell-secreted factors, e.g., lactic acid, TAMs undergo complex metabolic changes, especially by stimulating *de novo* lipid biosynthesis and becoming involved in several pro-tumoral functions [231]. Transcriptome and lipidomic analysis confirmed the upregulation of FA synthesis in human macrophages exposed *in vitro* to tumor cells [231]. Still, in this study, the macrophage contents of PC, PE, and sphingomyelins increased, and those of phosphatidic acid, lysophospholipids, and ceramides decreased. Another lipidomic analysis of TAMs in a model of gastric cancer reported increased *in vitro* uptake of extracellular lipids by M2-like TAMs generated in the presence of lipid-containing tumor explant supernatants [199]. M2-TAMs accumulated TG without changes in the levels of CE; moreover, such lipid-loaded TAMs were also present in clinical gastric cancer patients [199]. In summary, lipid droplets in TAMs appear to store mainly TG

with variable amounts of glycerophospholipids and sphingomyelins.

Lipid-laden TAMs can release lipids to the TME, where they can be taken up by cancer cells. Regarding TAM's ability to release cholesterol, RAW264.7 mouse macrophages are capable of transferring this lipid to prostate tumor cells and, moreover, infiltration of TAMs appeared to serve in providing cholesterol as the precursor molecule to promote the synthesis of intratumoral androgens, as it was observed *ex vivo* in patient-derived tumor explants after prostatectomy [232]. However, a specific tumor-promoting cholesterol efflux mechanism induced in macrophages by cancer cells, as also observed in a previous report on metastatic ovarian cancer [233], remained unclear. An earlier study in A-I transgenic and A-I-KO mouse models showed that apoA-I, but not apoA-II, through exerting specific modulatory effects on both innate and adaptive immune processes, induced a switch in the TAM phenotype from the pro-tumoral M2- to an antitumoral M1-type and resulted in melanoma tumor regression, suggesting a beneficial role of apoA-I in cancer therapeutics by redirecting *in vivo* elicited tumor-infiltrating TAMs toward tumor rejection [234]. Notably, it was recently demonstrated that an efficient cholesterol removal from cancer cells mediated by small discoidal reconstituted HDL particles resulted in decreased proliferation and induction of apoptosis of pancreatic ductal adenocarcinoma cells *in vitro* [235]. These findings suggest a therapeutic potential of ABCA1-mediated cholesterol depletion in pancreatic-cancer malignancy and likely point to a more general antitumoral effect of apoA-I via reduction of cholesterol in lipid rafts facilitated by HDL and preβ-HDL particles within the TME [236,237]. Also, in a mouse model of glioblastoma, high cholesterol levels in the TME impaired the phagocytic activity of TAMs, possibly by upregulating the "don't eat me" receptors; moreover, enhancement of cholesterol efflux from TAMs with the use of an apoA1-armed oncolytic adenovirus restored the macrophage antitumor immunity and elicited long-term tumor-specific immune surveillance [238]. These results provide further insight into the inherent mechanisms acting in malignant solid tumors by which cholesterol metabolism impairs antitumor immunity and offer a novel approach to the use of reconstituted HDL particles and recombinant apoA-I-expressing adenovirus for targeted manipulation of cholesterol efflux both in cancer cells and TAMs.

Overall, multiple transcriptional profiles of diverse TAM subpopulations have been observed in mice and human tumors as cancer progresses. Importantly, the complex lipid metabolism connections that occur between TAMs and distinct types of cancer cells, leading to lipid loading of TAMs influences a phenotype bias from M1 to M2, which ultimately promotes tumor occurrence and cancer development [239,240]. Fig. 2 **(top)** summarizes the interplay between lipid-loaded TAMs and cancer cells in a malignant tumor's hypoxic and acidic TME. Since, in contrast to the cancer cells, which experience little apoptosis, TAMs can engage in various regulated macrophage death processes, including apoptosis, necroptosis, and pyroptosis, it is plausible to infer that the balance between TAM proliferation and death is relevant for tumor growth. In this context, whether excessive lipid-loading of TAMs may lead to their apoptosis and death, such as in the atherosclerotic plaques, is unknown. Understanding the actual effect of TAM apoptosis on their pro- vs. anti-tumor functions in solid tumors remains a challenge and deserves further studies.

Finally, given the high plasticity of TAMs, their potential repolarization towards the M1 antitumoral phenotype opens new avenues for treating solid tumors, which may include dietary and pharmacological therapies [241]. In this regard, administering a high-fat diet to tumor-bearing mice was found to raise the abundance of lipid-loaded TAMs, suggesting stimulation of lipid-loading and the pro-tumoral functions of TAMs by dietary lipids [227]. This raises the question of whether modulating the lipid composition of the diet can lead to a potential M2/M1 TAM reverse conversion. Notably, TAM repolarization from the M2-to-M1 phenotype has been achieved with the use of nanoparticles targeted to provide macrophages with genes encoding master regulators of macrophage polarization, which leads to a program of antitumoral functions [242]. Moreover, a dual pH-sensitive nanodrug, which can repolarize TAMs for antitumor immunotherapy while minimizing the risk of breaking the M1/M2 polarization balance in healthy organs, has been used in a preclinical study [243]. These novel biotechnological advances highlight the immunotherapeutic potential of remodeling the immunosuppressive tumor capacity by modifying the gene profile of TAMs.

8. Lipid-loaded macrophages in the obese adipose tissue

All animal species store fat in the form of TG in the adipocytes of AT, a large and dynamic endocrine organ that can make up between 2–70 % of human body weight [244]. Although AT is present in various body locations, the metabolic derangements described in the human obese AT refer particularly to the accumulation of fat in the visceral AT rather than in the subcutaneous AT [245]. Chronic energy imbalance between calories consumed and calories expended leads to the excessive AT expansion characteristic of obesity and disrupts the AT homeostasis into a proinflammatory, hypoxic, and fibrotic state. In humans, two types of specialized AT exist: the white AT (WAT) and the brown AT (BAT), the percentage of the latter decreasing with age [246]. The uncoupling protein 1 and other programs of futile energy cycling allows BAT to burn excess energy, majorly derived from TG, to produce heat and act as a metabolic sink [247]. In contrast, WAT adipocytes have a large capacity for lipid storage in large lipid droplets, and they are capable of expanding their lipid cargo and so increasing their size to nearly 100 μm in diameter [248]. The plasticity of the AT was further highlighted by the reversible transdifferentiation of subcutaneous WAT in the mammary glandular tissue into alveolar epithelial cells known as pink adipocytes, so generating the pink AT capable of synthesizing and secreting milk [249,250]. Moreover, WAT browning has been observed in response to environmental changes such as it was observed in patients who underwent surgery for benign adrenal tumors [251].

The plasma lipid profile in obesity is characterized by elevated levels of FFA, TG-rich lipoprotein remnants, and LDL, and decreased levels of HDL [252,253]. The high concentrations of circulating albumin-bound FFA, particularly saturated FA, correlate with hepatic and muscle insulin resistance in obese adults [254]. Abdominal obesity, which is characterized by increased lipid accumulation in the visceral AT, enhances the expression of systemic proinflammatory cytokines like TNFα, which, together with high levels of palmitate, predispose to a dysfunctional endothelial barrier [255,256]. Thus, the obese AT is rich in FFA derived from an increased direct passage of systemic FFA through the AT vascular endothelium and from the lipolytic activity of LPL on TG on chylomicrons and VLDL, locally releasing FFA which, then enter the AT interstitial fluid enriching the FFA pool. Such FFA abundance in the obese AT stimulates adipocyte FA uptake and TG synthesis, particularly under insulin-deficient states [257]. An excessive load of TG in adipocytes initiates apoptotic signaling pathways, including the death receptor and mitochondrial pathways, which induce activation of effector caspases, resulting in adipocyte apoptosis [258]. However, hypertrophic adipocytes are also able to release FFA by intracellular lipolytic enzymatic processes orchestrated by adipose triglyceride lipase, hormone-sensitive lipase, and monoacylglycerol lipase [259]. Although, to our best knowledge, no reports are available on the pH value of the obese adipose tissue, a stoichiometric relationship between proton release and extracellular FFA concentration was demonstrated in rat adipocytes after TG breakdown *in vitro* [260]. Moreover, lactate production is selectively increased in adipocytes of obese AT where it functions as a paracrine signal to potentiate the polarization of ATMs to an inflammatory state [261]. Thus, it is likely that the high concentrations of FFA and lactate prevalent in obese AT can perturb the extracellular pH, resulting in a slight to moderate acidification of the interstitial fluid.

The AT also harbors non-adipocyte cell types, including macrophages, stem cells, fibroblasts, neutrophils, lymphocytes, mast cells, and

capillary endothelial cells [262]. A crosstalk between adipocytes and the network of non-adipocyte cells, especially macrophages, is key in supporting AT development and function [263]. Moreover, the macrophage platelet-derived growth factors family was found to be required for the regulation of energy storage in adipocytes [264]. The genetic characterization of ATMs was primarily derived from murine models of obesity, which indicated a wide variety of ATM phenotypes. ScRNA-seq studies have identified analogous ATM populations between humans and mice; however, the proportions of these cells differ in obese humans and mouse models [265]. The increased ATM number in the obese AT (40–60 % of the cell population, as compared to 10–15 % in the lean AT) is a consequence of their enhanced recruitment and local proliferation [266–268]. The ATM activation and metabolic phenotypes influence the development of obesity-induced metabolic disorders [269]. Profiling analysis of ATMs isolated from genetically- and dietary-induced obesity mouse models disclosed that at an early stage of AT expansion, when the glycemia is relatively preserved, ATMs express M2 anti-inflammatory markers. However, the chronic low-grade inflammation associated with insulin resistance is also related to the accumulation of M1-like proinflammatory macrophages in the obese AT. Different lines of evidence have approached the mechanisms linking AT expansion with ATM polarization. Among them, progressive lipid accumulation in CD36-expressing M2-ATMs induces a switch from the M2-like to the proinflammatory M1-like polarized ATMs, which is the phenotype associated with severe obesity and insulin resistance [270]. Interestingly, when exposed to high glucose levels *in vitro*, human macrophages undergo M1-type inflammatory polarization, with similar responses also being detected in circulating monocytes from prediabetic patients [271]. The inositol-requiring enzyme 1α (IRE1α), which is activated by obesogenic factors such as FA and LPS, has also been identified as a critical switch governing M1/M2 macrophage polarization, i.e., acting by repressing M2 while enhancing M1 polarization [272]. It was recently shown in murine models of obesity that LXRβ-deficiency, but not LXRα-deficiency, was responsible for the elevated expression of proinflammatory cytokines in macrophages, so highlighting the distinct roles for each LXR isoform in the regulation of AT inflammation [273]. This finding may provide important insights regarding the development of new therapeutic approaches in the treatment of obesity-induced

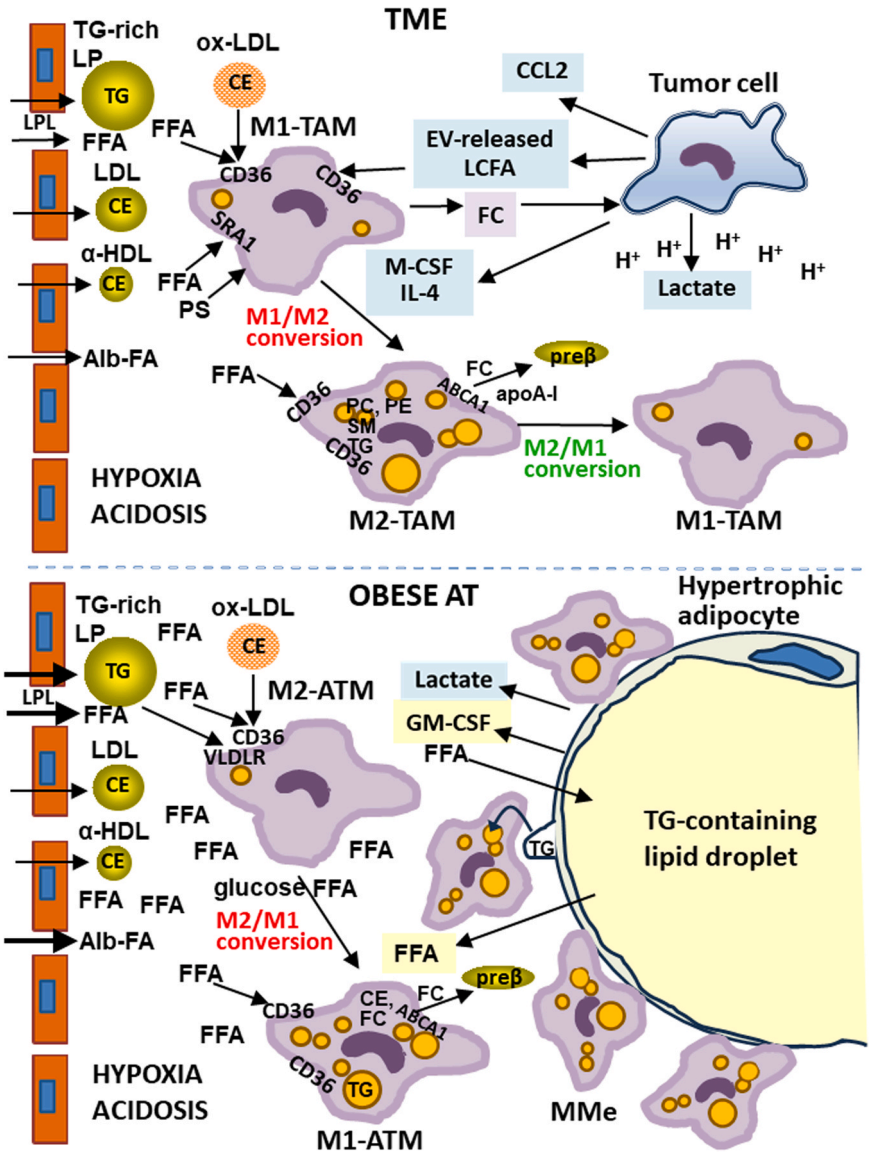

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Fig. 2. Cell-to-cell crosstalk of tissue-resident macrophages in the tumor microenvironment and in the obese adipose tissue generating lipid-loaded macrophage foam cells. Lipid-loaded macrophage populations of various phenotypes are formed both in cancer and obesity and they display metabolic cooperation with tumor cells and hypertrophic adipocytes, respectively. The TME and the obese AT contain a vast population of macrophages and share microenvironmental conditions, such as high levels of extracellular FFA, interstitial fluid acidosis, and hypoxia, which can influence the phenotype and lipid cargo of macrophages. TOP: Tumor cells secrete CCL2, which promotes macrophage recruitment. They also secrete M-CSF, IL-4, and other cytokines, which induce macrophage polarization towards the pro-tumoral M2-like TAMs in the hypoxic and lipid-rich TME. The high glycolytic rate of cancer cells increases the production of lactic acid, promoting lipid accumulation in neighboring TAMs and further inducing M2-TAM polarization. Uptake by TAMs of FFA derived from circulating Alb-FA and other lipoproteins present in the TME mainly occurs via enhanced CD36 expression, characteristic of the M2-polarized TAM phenotype. Lipid exchange occurs via the release of tumor cell-derived LCFA, which are carried by EV in tumor cells and can be taken by CD36 expressed in TAMs. Also, tumor cells can scavenge membrane cholesterol from TAMs and induce their reprogramming toward the M2 phenotype, promoting cancer progression. PS released from apoptotic tumor cells can also promote the transition of TAMs from M1 into M2 phenotype. In contrast, ABCA1-mediated cholesterol efflux to apoA-I and preβ-HDL can reverse the M2 phenotype towards the M1-TAM by reducing the abundance of lipid droplets. BOTTOM: Plasma lipids in obesity are associated with high Alb-FA and TG-rich lipoproteins, and low HDL levels. FFA are released from VLDL by the action of LPL and are captured in abundance by both adipocytes and ATMs. Hypertrophic adipocytes containing big fat droplets also release FFA through a lipase-dependent mechanism and, alternatively, by lipid-laden exosomes. Obese adipocytes release lactate further contributing to the AT acidification. Multiple ATM subpopulations with distinct functional phenotypes coexist in the obese AT, where they exert different impacts on obesity progression. In the lean AT, the M2-ATM phenotype is predominant, but exposure to excess glucose and FFA levels during the onset of obesity induces M1-ATM polarization. GM-CSF secreted by adipocytes of the obese AT may also promote macrophage recruitment and M1-ATM polarization. Due to steric limitations, ATMs aggregate surrounding large adipocytes, typically forming CLS at sites of adipocyte death. M1-ATM phenotype predominates in CLS and ATMs expressing MMe phenotype, which promotes the clearance of dead adipocytes through a process of lysosomal exocytosis, are also found in CLS from human AT. M1-ATMs overexpress ABCA1, CD36, and proinflammatory cytokines and promote dead adipocyte clearance through lysosomal exocytosis via acidic lysosomal synapses. The continuous influx into the obese AT of dietary lipids and FFA released by adipocytes promotes lipid uptake in ATMs mediated by CD36 and other scavenger receptors, further promoting the formation of lipid-loaded ATM foam cells. The increase in lipid-loaded ATMs is maximal at the peak adipocyte size, indicating that necrotic adipocyte debris is an important lipid source for ATMs. Lipid-loaded ATMs can also efflux cholesterol to preβ-HDL, alleviating inflammatory responses in the obese AT. *Abbreviations*: high-density lipoprotein (HDL), low-density lipoprotein (LDL), oxidized-LDL (ox-LDL), cholesteryl esters (CE), free cholesterol (FC), ATP-binding cassette transporter (ABC), cluster of differentiation 36 (CD36), scavenger receptor class A1 SRA1, tumor microenvironment (TME), interleukin, (IL), adipose tissue (AT), free fatty acids (FFA), monocyte chemoattractant protein-1 (CCL2), macrophage colony-stimulating factor (M-CSF), albumin-bound FA (Alb-FA), long-chain FA (LCFA), extracellular vesicles (EV), phosphatidyl serine (PS), lipoprotein lipase (LPL), granulocyte-macrophage colony-stimulating factor (GM-CSF), crown-like structures (CLS), metabolically activated (MMe), triglycerides (TG), triglyceride-rich lipoprotein (TG-rich LP), very-low-density lipoprotein receptor (VLDLR), tumor-associated macrophages (TAMs), adipose tissue macrophages (ATMs).

metabolic disorders, AT inflammation, and insulin resistance. Besides factors affecting obese AT biology, such as inflammatory conditions, adipocyte cytokine secretion could also affect macrophage polarization. The obesity-associated M1-like ATM phenotype is in line with early studies showing that expression of M-CSF was unchanged in genetic and high-fat diet obese mice models [274]. In contrast, elevated serum levels of the M1 polarization factor GM-CSF have been detected in diet-induced obesity in mice [275]. Moreover, a decreased number of macrophages was reported in WAT of GM-CSF$^{-/-}$ mice despite their higher amount of body fat, suggesting the role of this cytokine as a macrophage recruitment, besides its action inducing M1 polarization [276].

The model establishing a pathogenic transition from M2-to-M1 in the obese AT has continually evolved after introducing more accurate technologies in transcriptome analysis. Thus, multiple ATM subpopulations that respond distinctly to complex activation states *in vivo* have been identified, evoking mixed or unique phenotypes, such as the "mixed" M1/M2-ATM phenotypes described in obese mice and humans [277]. A metabolically activated (MMe) phenotype was induced by palmitate, glucose, and insulin, which can promote the clearance of dead adipocytes through a process of lysosomal exocytosis [278]. MMe-ATM subpopulation was characterized as a pro-tumoral phenotype, representing the predominant macrophage phenotype and an important source of IL-6 in the mammary obese AT of humans and mice [279]. In contrast to the classically activated ATMs expressing CD38, CD319, and CD274 cell surface markers, the MMe subtype from obese humans and mice overexpressed ABCA1, CD36, and proinflammatory cytokines [280]. This ATM phenotype is considered exclusive to metabolic disorders associated with obesity, such as insulin resistance and type 2 diabetes [281]. Furthermore, the high heterogeneity and plasticity of ATMs in the obese AT is supported by data derived from single-cell technologies, which have detected the absence of the classical M1 and M2 states but, instead, the presence of ATM subpopulations with a continuum state of cell phenotypes, being more proinflammatory or more metabolically active [282–284]. A conserved type of lipid-associated ATMs (known also as LAMs) driven by the macrophage lipid receptor Trem2 signaling has also been detected in mouse models of obesity and in visceral AT of obese, but not lean individuals [285]. Importantly, the lipid receptor Trem2 was identified as a major driver of AT macrophage beneficial responses during obesity, favoring a LAM program involving phagocytosis, lipid catabolism, and formation of ATM clusters surrounding hypertrophic and cell death-prone apoptotic adipocytes [285]. Studies in murine and human obesogenic conditions have shown that this LAM subpopulation is marked by expression of *Cd9* and Trem2, and in addition also expresses high levels of lipid handling genes, including *Cd36*, *Lpl*, and *Lipa* [286].

Large fatty adipocytes in the murine obese AT were reported to release FFA and induce a program of lysosome gene transcription and biogenesis in ATMs, which was associated with lipid catabolism but unrelated to the proinflammatory M1-like polarization [287]. It was further demonstrated that adipocytes release lipid-laden exosomes that become another source of TG for local ATMs and that AT from lean mice released ~1 % of its lipid content per day via exosomes *ex vivo,* and this rate increased by 2-fold in obese animals [288]. Obesity increases expression of the VLDL receptor (VLDLR) in AT and was reported a role of VLDLR in the onset of obesity by binding blood-derived extracellular VLDL and TG-rich lipoprotein remnants at the surface of both adipocytes and ATMs, so facilitating a subsequent TG uptake by these cells. Also, an elevated VLDLR expression in ATMs promoted proinflammatory responses in AT and glucose intolerance in obese mice; moreover, VLDL treatment upregulated intracellular levels of palmitate in ceramides in a VLDLR-dependent manner, which promoted M1-like ATM polarization [289].

The CD36 receptor also plays an important role in obese AT, facilitating the scavenging of FFA and other lipids released by adipocytes and lipoproteins derived from circulation by ATMs. When investigated in the CD36-KO mouse model, CD36 affected the function of AT, so leading to the conclusion that CD36 enhances adipocyte death in response to high-fat feeding and contributes to inflammatory responses in the AT by promoting proinflammatory cytokine expression both in adipocytes and in ATMs [290]. Beyond the canonical role of CD36 in scavenging pathological ligands, macrophage CD36 can promote macrophage apoptosis [291]. At the same time, its absence protects the CD36$^{-/-}$ mice from insulin resistance, which is associated with diet-induced obesity

and hyperlipidemia, while CD36 ligands, such as ox-LDL, mediate CD36-dependent inflammatory paracrine loops between adipocytes and their associated macrophages [292]. Lipid uptake by macrophages could be beneficial in lipid trafficking at the initial stages of obesity by helping adipocytes to buffer the increased amounts of FFA and other lipids entering the expanding AT. However, excessive lipid loading, inducing macrophage death and enhanced inflammation, could be detrimental in later stages.

A hallmark of the obese AT is the engulfment of dead adipocytes by ATMs. The relatively small size of macrophages is a steric impediment for ATMs to phagocytose hypertrophied fatty adipocytes. Consequently, multiple ATMs with extracellular lysosomal compartments surrounding hypoxic, necrotic, and dead adipocytes are the typical histological pattern of the obese AT in humans and mice [293]. More than 90 % of ATMs in the obese AT from both humans and mice form clusters localized around dead or dying adipocytes and display a metabolically activated phenotype, whereas in the lean AT, they are distributed evenly, interstitially spaced, and mainly show anti-inflammatory phenotypes [294,295]. The ATM clusters form the characteristic crown-like structures (CLS), a key feature of AT inflammation [296]. Studies in obese rodents have shown that, when compared to subcutaneous WAT, the CLS are prevalent in visceral fat, suggesting that visceral adipocytes are more susceptible to cellular death [297,298]. Importantly, LAMs are enriched at CLS, where they also appear to play an important and Trem2-dependent role in CLS assembly and overall AT remodeling during obesity [285]. Such lipid-loaded ATMs forming CLS in the obese setting contain abundant lipid droplets, recalling the foamy macrophages in atherosclerotic plaques at the light microscope. Deciphering the intricate signaling mechanisms that trigger the phagocytosis of dying adipocytes by ATMs might, hopefully, direct the phagocytic function of macrophages in the TME toward surrounding cancer cells.

Notably, the minor subclass of non-classical monocyte $CD14^{dim}CD16+$ circulating monocytes has been identified to be the cellular source of lipid-loaded ATMs, and, accordingly, the variable percentages of this monocyte subpopulation in blood were proposed to serve as putative predictors of visceral fat inflammatory state, as reflected by their particularly abundance in obese patients circulation and their high lipid content once they are converted into ATMs [299]. The percentage of lipid-loaded ATMs is increased in the visceral AT of obese ob/ob mice, consistent with an increased inflammatory state [270,300]. Lipid-loaded ATMs expressing CD9 + and localized within the CLS were also found to express proinflammatory transcriptomes and induce the inflammatory signature characteristic of the obese AT; moreover, the adoptive transfer of CD9 + ATMs was able to induce an obese-associated inflammatory state in lean mice [301]. Overall, both human and mouse ATMs from the obese AT significantly express CD36, TNFα, IL1β, and ABCA1, and moreover, a positive correlation between CD36 expression and BMI has been reported [280]. There is no evidence whether ABCA1-mediated cholesterol efflux to the abundant preβ-HDL population in the interstitial fluid can potentially reduce the lipid droplets in ATMs and so induce a reverse M1/M2-ATM conversion, such as it has been demonstrated in lipid-loaded TAMs.

High lipid contents in the CLS-forming lipid-loaded ATMs in human obesity have been detected by BODIPY-labeling, which stains neutral lipids, notably TG and CE [299,300,302]. However, only a few reports have been published on the specific composition of the ATM accumulated lipids. Instead of being filled with CE, as the macrophage foam cells of atherosclerosis, lipid droplets in ATMs are primarily loaded with TG, the major component also of the adipocyte lipid droplet, selectively enriched with saturated short-chain FA and small amounts of FC [270]. In mouse models of obesity, Abcg1 was markedly expressed in M2-ATM, suggesting their participation in the removal of FC from adipocytes via collision-based diffusion or by pinocytosis of dead adipocytes, followed by FC efflux to extracellular acceptors and so contributing to the homeostasis of FC in the obese AT [303]. The fact that lipid-loaded ATMs develop once adipocyte hypertrophy is maximal supports the view that the origin of the accumulated lipids may be related to ATM scavenger activity on dying nonviable adipocytes [304]. Overall, increased uptake of TG-rich lipoproteins and FFAs present in high levels in the obese AT, the internalization of adipocyte-derived exosomes by micropinocytosis, and the clearance of apoptotic TG-rich adipocyte debris by ATMs in advanced obesity are considered the main sources of TG which are primarily accumulated in lipid-loaded ATMs [281]. Mimicking the fate of hypertrophic adipocytes, excessive lipid accumulation in ATMs impairs their capacity for lipid handling. Eventually, it leads to macrophage apoptosis, contributing to increased inflammation and AT dysfunctionality in obesity. Fig. 2 **(bottom)** shows the crosstalk between ATMs and adipocytes within the hypoxic and lipid-rich obese AT, which generates lipid-loaded ATMs.

9. Concluding remarks

The vast population of tissue-resident macrophages in humans modulates key physiological and pathological events in organs and tissues by producing cytokines, growth factors, and signaling molecules. Despite the recent advances in our understanding of macrophage plasticity, there are still many unanswered questions about how macrophage subtypes are generated depending on their localization and microenvironmental conditions. As described in the previous sections, abundant macrophage populations, high levels of extracellular lipids, acidity, and hypoxia are common characteristics of the ECM of an atheroma, a malignant tumor, and an obese AT. Notably, the role of lactate (secreted by macrophages, tumor cells, and obese adipocytes) has been recently highlighted as a signaling molecule in human disease by promoting development of the atherosclerotic plaques, certain malignant tumors, and obesity [305]. In addition, the distinct mixtures of cytokines and growth factors around tissue-resident macrophages are also capable to operate in concert modifying their polarization in these body compartments, and further stimulating a dynamic phenotype reprograming. All these signals also interact with the lipid-rich environment and, notably, a single action or the intensity of any of them is likely able to completely govern the continuous process of macrophage polarization.

Given the abundance of macrophages filled with lipid droplets associated with multiple metabolic disorders, such as atherosclerosis, cancer, and obesity, the pathophysiological involvement of macrophage lipid metabolism has recently gained much attention in clinical-oriented studies. Current knowledge has established that embryonic resident tissue macrophages are ontogenically distinct from the later adult bone marrow-derived monocytes. Although each tissue has its unique hybrid composition of embryonically- and adult-derived macrophages, it is still unclear whether the macrophage origin dictates their ability to become lipid-loaded cells, thus affecting their functionality in human diseases.

Macrophage polarization *in vivo* is more complex than the traditional M1/M2 model described by *in vitro* studies. Their functional plasticity allows them to metabolically respond to changes in their living microenvironments, and via crosstalk with ECM components and neighboring cells, multiple phenotypic subpopulations of tissue-resident macrophages are generated. Notably, scRNA-seq data have disclosed a wide spectrum of macrophage clusters and genes in *ex vivo* tissues, e.g. in specimens of tumors and atherosclerotic plaques. However, there are still many challenges and limitations in the application of these emerging technologies, such as the sample availability, the optimal cell survival in human tissues obtained post-surgery, and the variation in processing protocols to digest the tissues [56]. These novel technologies will open the gate for precision or personalized medicine based in patient-specific macrophage profiling to tailor the right quality disease treatment for the right patient.

Among other factors, the occurrence of lipid disorders affecting the contents of lipids in the interstitial fluids surrounding macrophages contributes to modulating their polarization and functional properties. The commonly used M1/M2 broad classification is based on the immunological properties and expression of specific macrophage

markers; therefore, the M1 and M2 macrophages identified in atherosclerotic plaques, malignant tumors, and obese AT are not phenotypically identical. Then, it is not surprising that the M2 phenotype, generally described as being the one involved in tissue repair and regeneration, is actually the polarized macrophage subtype that supports foam cell formation in atherosclerotic lesions and the TME, but not in the obese AT. The intricate interplay between macrophages and their tissue microenvironments and neighboring cells can also modify their metabolic profiles, allowing them to adapt to hypoxia and nutrient deprivation, leading to functional reprogramming of their phenotypes.

High circulating levels of albumin-bound FA and plasma lipoproteins, particularly high LDL and low HDL, induce lipid-loaded macrophage subpopulations in various body compartments that modify macrophage phenotype and functions. Multiple lines of evidence have described adverse functional responses of macrophages containing large dysfunctional lipid droplets in the genesis of atherosclerotic lesions, malignant tumors, and the obese AT. Those body compartments share several common characteristics, such as impaired vascularization, hypoxia associated with decreased blood supply, acidosis, increased vascular permeability, and lipid-rich interstitial fluids. Over the decades, novel molecular mechanisms involved in foam cell generation and their central role in atherosclerosis have been disclosed, including the functions of nuclear receptors, non-coding RNAs, and gut microbiota [96]. However, the specific definition of macrophage phenotypes driving human diseases is still challenging because the existing data is primarily based on transcriptomics analysis performed in genetically modified animal models, mainly rodents. Discordance in the published data may be attributed to variations in the advanced state of the studied disease and the use of specific microenvironmental conditions, genetic or diet-induced animal models, and different experimental methods. In general, based on immunohistochemical staining of macrophage markers and other data on human tissue-resident macrophages, cholesterol-laden M2-like polarized macrophages appear to occur in greater numbers in atherosclerotic lesions, and another type of lipid-loaded M2-like TAM phenotype predominates in malignant tumors. In contrast, lipid-loaded M1-like ATM is the main phenotype that populates CLS formed around dying adipocytes in the obese AT. The time course to generate these macrophage phenotypes, whether polarization precedes lipid-loading or vice versa, may depend on pathophysiological conditions associated with the distinct metabolic disorder, like activation of the macrophage phagocytosis function. In any case, excessive uptake of neutral lipids and their storage in lipid droplets may ultimately lead to macrophage damage and death. Thus, understanding the dynamic microenvironmental changes in tissues in which lipid-laden macrophages reside appears to be crucial to developing novel clinical therapies and improving the therapy responses of individuals affected by macrophage-related diseases.

Based on the inverse association of LDL and HDL with atherosclerosis, basic therapeutic approaches have focused on decreasing LDL or increasing HDL levels. In contrast to the rapid development of efficient LDL-cholesterol-reducing drugs [306], clinical trials to increase HDL levels in patients have been challenging and have not yielded beneficial outcomes, as was anticipated, in patients with cardiovascular disease risks [307–309]. Nevertheless, it should be emphasized that chemical and enzymatic lipoprotein modifications (e.g., oxidation, proteolysis, lipolysis, fusion, and aggregation) potentially taking place in living tissues affect lipoprotein composition, size, and biological functions and, more importantly, generate dysfunctional lipoproteins. Such modifications increase the foam cell-forming potential of LDL and reduce the cholesterol efflux- and M-RCT-promoting capacities of HDL. Thus, rather than the mere quantities of LDL and HDL particles in blood (or in the optimal case in peripheral interstitial fluids), evaluating their actual functional qualities must be of major clinical relevance. The development of novel methods to measure lipoprotein functionality may allow us to incorporate more efficient biomarkers into clinical practice to predict the risk of cardiovascular disease and other lipid-related metabolic disorders, such as those associated with the generation of cholesterol-laden macrophage foam cells. Similarly, emerging strategies to exploit macrophages as therapeutic tools in various medical fields highlight the notion that, besides the quantity of macrophages accumulated in specific tissues, their functional quality is of utmost importance. It is worth mentioning that regulation of the diet composition, particularly avoiding the toxicity of high-fat diets, appears to be an important therapeutic player in preventing atherosclerosis, cancer, and obesity development.

In summary, one important characteristic of atherosclerosis, cancer, and obesity is the critical contribution of lipid-loaded macrophages to the development of these diseases under various stages. Yet, it is unknown whether the distinct subpopulations of polarized macrophages present in the TME, obese AT, and the atherosclerotic intima in patients concurrently affected by these pathologies would interact and phenotypically modify each other within the same individual. Moreover, the extensive identification of lipid-loaded macrophages in other metabolic disorders and infectious diseases calls for further research to be focused on the multiple, even opposing effects of macrophage foam cells in many human diseases. Besides atherosclerosis, this approach is gaining particular attention in the fields of cancer and obesity research [310, 311] but deeper exploration of the tumorigenic and obesogenic properties of such specific lipid-loaded macrophages still needs more systematic studies. Overall, understanding the molecular mechanisms of the epigenetic and transcriptional regulation of macrophages and identification of their interactions with neighboring cells in the respective lipid-enriched tissues, potentially fueled by artificial intelligence applications, will help the design of more precise dietary and pharmacologic strategies to prevent and treat the metabolic disorders behind atherosclerosis, cancer, and obesity, the three major global causes of disease and death.

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CRediT authorship contribution statement

Miriam Lee-Rueckert: Conceptualization, Writing – original draft, Writing – review & editing. Matti Jauhiainen: Conceptualization, Writing – review & editing. **Petri Kovanen:** Conceptualization, Writing – review & editing. **Joan Carles Escolà-Gil:** Conceptualization, Funding acquisition, Writing – review & editing.

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All authors declare that they have no relationships relevant to the contents of this paper to disclose and have approved the final version of the article.

Data availability

No data was used for the research described in the article.

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