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Review

Triacylglycerol uptake and handling by macrophages: From fatty acids to lipoproteins

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A B S T R A C T

Macrophages are essential innate immune cells and form our first line of immune defense. Also known as professional phagocytes, macrophages interact and take up various particles, including lipids. Defective lipid handling can drive excessive lipid accumulation leading to foam cell formation, a key feature of various cardiometabolic conditions such as atherosclerosis, non-alcoholic fatty liver disease, and obesity. At the same time, intracellular lipid storage and foam cell formation can also be viewed as a protective and anti-lipotoxic mechanism against a lipid-rich environment and associated elevated lipid uptake. Traditionally, foam cell formation has primarily been linked to cholesterol uptake via native and modified low-density lipoproteins. However, other lipids, including non-esterified fatty acids and triacylglycerol (TAG)-rich lipoproteins (very low-density lipoproteins and chylomicrons), can also interact with macrophages. Recent studies have identified multiple pathways mediating TAG uptake and processing by macrophages, including endocytosis and receptor/transporter-mediated internalization and transport. This review will present the current knowledge of how macrophages take up different lipids and lipoprotein particles and address how TAG-rich lipoproteins are processed intracellularly. Understanding how macrophages take up and process different lipid species such as TAG is necessary to design future therapeutic interventions to correct excessive lipid accumulation and associated co-morbidities.

1. Introduction

Macrophages are key cells of our innate immune system and represent one of the frontlines in the host's defense against pathogens. They specialize in the detection, engulfment, and subsequent degradation of bacteria and other harmful organisms through the process of phagocytosis. In addition, macrophages can present antigens and regulate inflammation by releasing cytokines. Besides taking up exogenous material such as pathogens, macrophages can also internalize different types of lipids, including fatty acids, phospholipids, cholesterol, and entire lipoprotein particles.

In macrophages and many other types of cells, fatty acids are oxidized to generate ATP [1]. By providing fuel to macrophages, fatty acids support basic macrophage functions such as phagocytosis, cytokine secretion, and antigen presentation, thereby contributing to immune and metabolic homeostasis [1,2]. The uptake of lipids by macrophages is a normal physiological process that occurs in numerous tissues, including the adipose tissue, liver, and lung. However, in several metabolic disease states such as atherosclerosis [3], non-alcoholic fatty liver disease [4], and obesity [5], lipid uptake by macrophages exceeds the capacity of macrophages to dispose of the lipids, leading to the appearance of lipid-laden or foamy macrophages that may contribute to disease progression. For example, in the vascular wall, macrophages take up oxidized LDL leading to foamy macrophages, which is a key event in the development of atherosclerosis [6]. In the liver, during the development of non-alcoholic fatty liver disease, the Kupffer cells internalize fatty acids and lipoproteins, including low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL), similarly leading to the formation of foamy macrophages [7–10]. In obese adipose tissue, macrophages surround dying adipocytes and take up fatty acids and triacylglycerol (TAG), thereby developing into foam cells [11]. These foam cells contribute to the inflammatory phenotype of obese adipose tissue [12], which in turn may play a role in the development of insulin resistance [13–15], although conflicting data exist [16]. Whereas foam cell formation is often viewed as a pathophysiological and detrimental hallmark of a diseased state, macrophage lipid storage, by mitigating the potential lipotoxic effect of a lipid-rich environment and associated elevated lipid uptake, can also be viewed as protective.

Currently, there is a lack of insight into the pathways involved in the uptake of certain types of lipids by macrophages, thus limiting our

Abbreviations: VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; TAG, triacylglycerols; LPL, lipoprotein lipase; LAL, lysosomal acid lipase; LDLR, low-density lipoprotein receptor; ATGL, adipose triglyceride lipase.

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understanding of the mechanisms leading to the appearance of foamy cells. Understanding how macrophages take up different lipid species at different locations is expected to generate novel mechanistic insights into the development of the abovementioned diseases.

In recent years, the mechanisms underlying the uptake and intracellular processing of (modified) LDL particles by macrophages have become increasingly clear. In contrast, our understanding of the pathways facilitating the uptake and processing of other lipids, including fatty acids and the TAG-rich lipoproteins VLDL and chylomicrons by macrophages, remains sketchy. Multiple pathways are likely involved in the uptake of a wide range of lipid species. For example, the uptake of fatty acids has been shown to involve various pathways ranging from passive transport to receptor-mediated internalization [17]. Compared to fatty acids, lipoproteins are more complex structures and thus require different mechanisms for uptake. For example, lipoprotein uptake and utilization by cells may require TAG hydrolysis by lipases leading to the release of fatty acids that are subsequently taken up by cells. Other lines of evidence support an important role for scavenger receptors, such as CD36 and SR-A (MSR1, CD204), in mediating lipoprotein uptake [18]. Endocytosis, a pathway used by macrophages to take up extracellular material, has also been linked to lipoprotein uptake [19,20]. Clathrin-mediated endocytosis, often referred to as receptor-dependent endocytosis, is responsible for lipid uptake via transmembrane receptors and transporters and occurs through clathrin-coated pits formed by the assembly of the major coat proteins called clathrin triskelia [21]. Caveolae-dependent endocytosis, also called raft-dependent endocytosis, is defined by its clathrin independence, dynamin dependence, and sensitivity to cholesterol depletion and also contributes to lipid uptake by different cell types [22–24].

Here, we will review the current literature on how macrophages take up different lipid species ranging from non-esterified fatty acids (NEFA) to more complex lipid and lipoprotein structures. In addition, we discuss how internalized TAG-rich lipoproteins are processed inside macrophages. The intracellular processing of cholesterol will not be addressed in detail here, as it has already been covered by several recent review papers [25–28].

2. Sources and mechanism of lipid uptake and accumulation in macrophages

2.1. Non-esterified fatty acids

NEFA, which can originate from lipolysis of TAG stored in the adipose tissue, are taken up by macrophages using multiple pathways, including transporter-mediated uptake and endocytosis.

2.1.1. Fatty acid uptake by transporters

It is well-established that specific transporters or receptors facilitate fatty acid uptake in cells, including macrophages. An important transporter of fatty acids in macrophages is CD36. CD36 is part of a class of transporters belonging to the group of Pattern Recognition Receptors. These receptors play crucial roles in the innate immune system by recognizing specific structures of pathogens leading to immune cell activation [29–31]. CD36 expression increases as monocytes differentiate into macrophages [32,33], thereby allowing for an increased uptake of fatty acids. Macrophage CD36 expression is stimulated by macrophage colony-stimulating factor and interleukin 4 [34], which are cytokines promoting the differentiation of alternatively activated macrophages. Alternatively activated macrophages play a role in resolving pro-inflammatory responses and promoting tissue repair [35]. These findings may suggest differences in fatty acid uptake capacity depending on the function and phenotype of the macrophage. Interestingly, unsaturated fatty acids induce CD36 gene expression in human macrophages in a dose-dependent manner [36], suggesting that fatty acids upregulate the mechanisms required for their cellular uptake. Overall, however, we still miss a thorough understanding of whether CD36 is indispensable for fatty acid uptake by macrophages, as well as of the association between CD36-mediated fatty acid uptake and the functional phenotype of the macrophage.

In addition to CD36, fatty acid transport proteins FATP1 and FATP4 are expressed in macrophages [37]. FATP1 deficiency was shown to significantly reduce fatty acid uptake by bone marrow-derived macrophages, whereas overexpression of *Fatp1* in RAW 264.7 macrophages caused a 17% increase in fatty acid uptake [37]. The deficiency of FATP4 in bone marrow-derived macrophages led to altered levels of numerous lipid species, including various phospholipids and sphingolipids, which were highly dependent on the type of macrophages and the specific treatment. Interestingly, FATP4 deficiency was not associated with a decrease in macrophage TAG content. Unfortunately, the impact of FATP4 deficiency on NEFA uptake has not been studied [38].

2.1.2. Fatty acid uptake by endocytosis

In addition to uptake via specific transporters, adipocytes [39], liver cells [40], and endothelial cells [41]have also been suggested to take up fatty acids via caveolae-mediated endocytosis or macropinocytosis. Although endocytosis and macropinocytosis are crucial for macrophages to internalize various particles, including viruses, bacteria, and soluble nutrients, their role in mediating fatty acid uptake by macrophages remains unclear [42]. Future studies should investigate the involvement of different endocytosis pathways, such as caveolae-mediated, clathrin-mediated endocytosis, and macropinocytosis, in fatty acid uptake by macrophages, possibly through genetic manipulation of specific components in the respective pathways.

2.2. (Modified) low-density lipoprotein (LDL)

LDL is a lipoprotein composed of apoB-100, phospholipids, unesterified cholesterol, cholesteryl esters, and TAG. Various macrophages, including Kupffer cells and macrophages in the vascular wall, are exposed to LDL particles. LDL can be modified by oxidation, acetylation, and glycosylation, which is tightly associated with the foamy change of macrophages [43]. The mechanisms involved in the uptake by macrophages of native or modified LDL, which include acetylated LDL (Ac-LDL) and oxidized LDL (Ox-LDL), are well-established and are elaborated on below [17,44–47]. The uptake of various LDL particles by macrophages may require the transport of LDL across the endothelium. Due to the size of LDL, transendothelial transport of LDL is mainly via the transcellular pathway referred to as transcytosis [48,49].

2.2.1. Lipoprotein lipase-mediated uptake

Lipoprotein lipase (LPL) is an extracellular and partly intravascular lipase that hydrolyzes the TAG contained in TAG-rich lipoprotein particles into fatty acids. LPL consists of two domains: a larger N-terminal catalytic domain and a smaller C-terminal domain that mediates the binding of lipoproteins. Initially, it was considered that the catalytic function of LPL controls LDL uptake by macrophages [44,50]. According to this concept, TAG in LDL particles are first hydrolyzed by LPL, and the resulting fatty acids are subsequently taken up by cells. However, several studies have suggested a different role of LPL involving its non-catalytic C-domain. In this scenario, LPL serves as a bridge between lipoproteins and heparan-sulfate proteoglycans and aids in the internalization of lipoprotein particles [45–47,51]. In macrophages derived from THP-1 cells, a human leukemia monocytic cell line, LPL increased the binding and uptake of LDL particles, but different from endothelial cells, the effect was independent of the LDL receptor [52]. The non-enzymatic involvement of LPL has also been reported in LDL receptor (LDLR)- or LDLR-related protein-independent uptake of glycated LDL, which is produced by a nonenzymatic reaction of glucose and LDL and is a preferred target of LDL oxidative modification [44]. While these studies suggest the involvement of the C-terminal part of LPL, the physiological relevance of the catalytic N-terminal part of LPL in LDL uptake by macrophages remains unclear. Accordingly, more research is

needed to determine the respective roles of the catalytic and non-catalytic functions of LPL in macrophage LDL uptake. It could be envisaged that the relative importance of the catalytic and non-catalytic function of LPL may depend on the local environment, such as the local presence of lipids and other nutrients, and the specific macrophage phenotype.

2.2.2. Receptor-mediated uptake

Many lines of evidence suggest that LDL undergoes oxidation in vivo. The oxidation-specific epitopes serve as Damage-Associated Molecular Patterns and are recognized by scavenger receptors that belong to the family of Pattern Recognition Receptors [53]. The scavenger receptors that mediate the uptake of Ox-LDL and cholesterol by macrophages and promote foam cell formation include LOX-1, CD36, and SR-A, as well as the non-scavenger receptor LDLR [7,54–59]. Upon binding the lipoprotein particles, the receptor is internalized and can be recycled back to the cell surface after lysosomal digestion of the lipoprotein content [60]. Monoclonal antibodies against CD36 inhibited the binding of Ox-LDL by 50% and the degradation of internalized Ox-LDL by 26% in human monocyte-derived macrophages [61]. In line with these findings, reduced uptake of Ox-LDL was found in human monocyte-derived macrophages lacking CD36 [59], demonstrating that CD36 participates in Ox-LDL uptake by human macrophages. Besides CD36, SR-A has also been reported to mediate the binding and degradation of Ac-LDL and Ox-LDL in macrophages [60,62]. Foam cell development after ex-vivo incubation of macrophages with Ox-LDL was significantly decreased in peritoneal macrophages isolated from SR-A and CD36-deficient mice compared to wild-type mice [60]. Studies in CD36-deficient mice showed that CD36 retards the clearance of native LDL and accelerates the clearance of Ox-LDL, suggesting that CD36 plays a significant role in Ox-LDL uptake [63]. Many studies performed in mice, though not all, have found that SR-A and CD36 contribute to atherosclerosis development [64–70]. However, it is still unclear which of the different scavenger receptors plays the most prominent role in the uptake of the modified LDL by macrophages and whether this may depend on the activation status of macrophages.

2.2.3. Clathrin-mediated endocytosis

Caveolae-mediated and clathrin-mediated endocytosis are two major types of endocytosis in macrophages. Although the role of caveolae-mediated endocytosis in macrophages is unclear, endocytosis mediated by clathrin has been well-studied in these cells [71]. Specifically, the internalization of Ox-LDL and Ac-LDL by THP-1 monocyte-derived macrophages was shown to be mediated by clathrin-mediated endocytosis [19]. Internalization is preceded by the binding of modified LDL to specific receptors such as LDLR and the scavenger receptors SCARB1, CD36, and SR-A [18]. Consistent with the uptake of LDL via clathrin-dependent endocytosis, electron microscopy showed the presence of LDL on clathrin-coated pits in primary human macrophages [72]. After endocytosis and subsequent formation of endosomes, the endosome fuses with the lysosome, followed by intracellular digestion of the lipid content of LDL by lysosomal acid lipase (LAL) and transfer of the resulting fatty acids and cholesterol to other cellular compartments [73,74].

2.2.4. Macropinocytosis

Macropinocytosis is an actin-driven and frequently occurring endocytic process in macrophages for the non-specific uptake of fluid into large cytoplasmic vesicles [75]. There is evidence that macropinocytosis is also involved in native LDL, Ac-LDL, and Ox-LDL uptake by macrophages [19] [76–81]. Interestingly, pathogen-derived molecules like lipopolysaccharide can significantly enhance macropinocytosis [82], suggesting that inflammatory activation may also activate macrophage macropinocytosis and thus LDL uptake [83]. Recently, it was shown that genetic or pharmacological inhibition of macropinocytosis reduces atherosclerotic lesion development in atherosclerosis-prone mice. Furthermore, stimulation of macropinocytosis promoted the macrophage uptake of native LDL, Ac-LDL, and Ox-LDL, and enhanced foam cell formation, which was independent of CD36 and SR-A [84].

2.3. TAG-rich lipoproteins (VLDL, chylomicrons)

TAG are predominantly transported by TAG-rich lipoproteins, which can either originate from the intestine or the liver. Chylomicrons have a diameter of 75-600 nm, contain dietary TAG, and are produced by enterocytes post-prandially [85]. VLDLs have an average diameter of 30-80 nm, are mainly synthesized by hepatocytes, and thus mostly contain TAG produced in the liver [86,87]. Only lipoprotein particles below ~70 nm diameter—which thus excludes non-lipolysed chylomicrons and very large VLDL but includes VLDL and VLDL remnants—can traverse the endothelium by active transcytosis and can be retained in the subendothelial layer of the artery wall. Consequently, macrophages can potentially interact and take up VLDL/VLDL remnants retained in the intima [88]. These VLDL/VLDL remnant particles contain the so-called remnant cholesterol, are considered to be highly atherogenic, and likely underlie the causal association between plasma TAG, TAG-rich lipoproteins, and their remnants on the one hand, and increased risk of atherosclerotic cardiovascular disease on the other hand [89]. By contrast, the interaction between macrophages and chylomicrons seems to be restricted to the mesenteric lymph nodes and *lamina propria*, where resident macrophages can directly interact with chylomicrons secreted by the enterocytes [90].

2.3.1. Lipoprotein lipase-mediated uptake

Already several decades ago, it was shown that LPL-catalyzed lipolysis is not required for the uptake of VLDL-TAG by cultured macrophages but does have a modest stimulatory effect [91,92]. Indeed, VLDL uptake by macrophages occurs in the absence of the LPL activator apolipoprotein C2, suggesting a lipolysis-independent pathway of VLDL-TAG uptake. Recently, using antibodies directed against the N- or C-terminal parts of LPL, we validated that the uptake of TAG contained in VLDL or chylomicrons by human primary monocyte-derived macrophages is dependent on the non-catalytic part of LPL but not on the catalytic function of LPL [20]. Collectively, it suggests that LPL's role in macrophage uptake of VLDL is more as a receptor or molecular bridge than as an enzyme. It is conceivable that LPL in macrophages may be largely enzymatically inactive due to the absence of an activator such as apolipoprotein C2 [93].

2.3.2. Endocytosis-mediated uptake by macrophage

The pathway by which macrophages internalize TAG-rich lipoprotein particles has remained largely unknown. We recently reported that mouse RAW 264.7 macrophages and human primary macrophages differentiated using Granulocyte-Macrophage Colony-Stimulating Factor take up VLDL and chylomicron particles via caveolae-mediated endocytosis and independently of clathrin-mediated endocytosis [20]. The uptake pathway appears to be mainly mediated by *CAV2*, one of the major caveolae-encoding genes. Since caveolae are 50-100 nm membrane micro-invaginations associated with the plasma membrane, it can be envisioned that the efficiency of uptake of TAG-rich particles by macrophages may depend on particle size [94]. It is still unclear to what extent the pathway of lipid uptake may depend on the type and activation state of macrophages and to what extent there is a difference between tissue-resident macrophages and Granulocyte-Macrophage Colony-Stimulating Factor- or Macrophage Colony-Stimulating Factor-stimulated monocyte-derived macrophages cultured ex vivo.

2.4. Lipids derived from apoptotic cells

Macrophages are professional phagocytes of endogenous apoptotic cells. This process of efferocytosis is important for maintaining cell and tissue function through the timely removal of non-functional cells.

Efferocytotic engulfment of dying cells by macrophages leads to the uptake of lipids, which can be used as fuel and polarize macrophages to aid in tissue repair [95]. Several receptors are involved in the recognition of apoptotic cells, such as SR-A, which has been suggested to be involved in the clearance of apoptotic thymocytes [96], and C-type lectin receptor LSECtin [97]. Other receptors likely involved in the phagocytosis of apoptotic cells by macrophages include CD36, MERTK, CD14, and FcγR [98].

In adipose tissue, macrophages are constantly confronted with lipids originating from adipocytes, mainly in the form of NEFA generated by lipolysis catalyzed by adipose triglyceride lipase (ATGL, PNPLA2) and hormone-sensitive lipase. During obesity and subsequent adipocyte expansion and cell death, macrophages accumulate around dying adipocytes to clear the intracellular lipid content [99]. It has been shown that macrophages form hydrolytic extracellular compartments at contact sites with dying adipocytes using local actin polymerization, leading to the uptake and degradation of fragments of apoptotic adipocytes [100]. Currently, it is unknown how these phagocytosed fragments are processed, whether the products of processing can be stored in the cells, and how this would impact macrophage function and phenotype [101].

Besides NEFA and apoptotic bodies or fragments, lipids contained in adipocytes may also travel and be taken up by macrophages as part of exosomes. Exosomes are tiny vesicles surrounded by a bilayer lipid membrane. They represent a transport vehicle for communicating between cells and harbor a variety of different cargo, ranging from various types of nucleic acids to proteins and lipids. Exosomes released by adipocytes contain various neutral lipids [102]. Interestingly, adipocyte-released exosomes injected into the gonadal adipose tissue of mice were taken up by adipose tissue macrophages. In addition, the treatment of cultured macrophages with adipocyte-released exosomes increased TAG accumulation, which did not require the activity of the esterifying enzyme DGAT. These findings indicate that adipocyte-released exosomes may contribute to lipid accumulation in adipose tissue macrophages, and suggest that the internalized TAG in the exosomes can be directly taken up and stored [102]. Possibly, the uptake and storage of adipocyte-released exosomes may be part of the lipid buffering function of adipose tissue macrophages [103].

3. Intracellular handling of internalized TAG-rich lipoproteins

After being internalized by macrophages, TAG need to be properly dealt with through intracellular processing, which includes lysosomal degradation, fatty acid trafficking, fatty acid oxidation, and lipid storage. How TAG are processed is coupled to the function of macrophages, as elaborated below. The intracellular processing of cholesterol has been addressed in recent reviews and will not be covered [25–28].

3.1. Relationship between lipid metabolism and macrophage functions

There is growing evidence that the functional properties of immune cells and their immunological phenotype are closely associated with how these immune cells produce energy, i.o.w. by their metabolic profile. For example, glycolysis is highly active in inflammatory M1-like macrophages providing both ATP and building blocks to support their inflammatory response [104,105]. M1 macrophages are classically activated, typically by interferon γ or lipopolysaccharide, and produce proinflammatory cytokines, phagocytize microbes, and initiate an immune response. By contrast, fatty acid oxidation has been associated with interleukin 4-induced M2 polarization [106]. M2 macrophages are alternatively activated, typically by interleukins 4, 10, or 13, and are involved in wound healing and tissue repair. Furthermore, lysosomal lipolysis-related fatty acid oxidation was shown to be essential for M2 activation [107]. However, it was also observed that macrophages with blocked β-oxidation of fatty acids were still fully polarized toward an M2 state after stimulation with interleukin 4 in vitro and in vivo, suggesting that fatty acid oxidation is largely dispensable for interleukin 4-driven M2 polarization [108]. Accordingly, the requirement for fatty acid oxidation in M2 polarization of macrophages might be more complex than previously envisioned.

Beyond macrophage polarization, several studies have also indicated that inhibition or enhancement of fatty acid oxidation influences the inflammatory properties of macrophages [106,108–112]. However, the results may be partly skewed by the activity of the β-oxidation inhibitor etomoxir toward other cellular pathways [113].

3.2. Lysosomal degradation and efflux

After the endocytosis of lipoproteins, the endosomes carrying the lipid particles fuse with lysosomes. The lysosomal enzyme LAL catalyzes the hydrolysis of various types of lipids including TAG and cholesterol esters [114–116]. Besides its role in the degradation of LDL, LAL also plays an important role in the intracellular processing of TAG-rich lipoproteins in macrophages, as shown by the accumulation of neutral lipids upon inhibition of LAL in human primary macrophages treated with TAG-rich lipoprotein particles [20]. Furthermore, LAL inhibition significantly decreased the levels of fatty acids in the culture medium. Together, these data suggest that lysosomal processing is a key determinant of the internal processing of lipids contained in lipoproteins but also the efflux of lipids in the form of NEFA [20].

The mechanisms involved in cellular trafficking and efflux of internalized TAG in macrophages were generally not very well-established. Recently, observations were made in human primary macrophages treated with VLDL and chylomicrons. It was shown that the lysosomal membrane protein STARD3 transports the VLDL-derived fatty acids directly from the lysosome to the ER [20]. Whether STARD3 directly interacts with fatty acids or stimulates transport via indirect pathways remains unclear. In the same study, NPC1 was shown to mediate the release of fatty acids derived from engulfed lipoproteins, likely via an indirect mechanism [20]. However, if other popular intracellular lipid transporters, such as NPC2 or VAP, are also involved in the process remains unclear. Overall, the specific mechanisms involved in intracellular fatty acid transport and the export of lysosomal-derived NEFA deserve further investigation.

3.3. Fatty acid oxidation versus storage

As discussed above, after lipid uptake by macrophages, fatty acids are transported out of the lysosome and undergo different metabolic fates, including β-oxidation to provide energy [117], esterification with glycerol-phosphate to form TAG for lipid storage [118], and excretion out of the cells [20]. Currently, the regulatory mechanisms driving the different metabolic fates of fatty acids remain poorly characterized yet are vital to understanding the physiological and pathological role of the internalized lipids for macrophages.

Beta-oxidation allows macrophages to harness the energy contained in fatty acids [119,120]. It depends on the import of fatty acids into the mitochondria, which is mediated by carnitine palmitoyl transferase 1 and 2 [108]. In many studies, macrophage metabolism is studied in in vitro settings using cell culture media high in glucose. In these settings, macrophages have been shown to mainly consume glucose as an energy source. Additional studies are needed to specifically assess the ability of macrophages to metabolize fatty acids. Also, to what extent macrophages rely on fatty acids as an energy source under normal in vivo situations is unclear.

3.4. Triglyceride storage and autophagy

Like nearly all cells, macrophages can store fatty acids as TAG in specialized lipid-filled vacuoles called lipid droplets. Lipid droplets are highly dynamic cellular organelles regulated by the balance between TAG storage and breakdown [20,121]. To enable their storage as TAG, the fatty acids generated by LAL-mediated hydrolysis first need to be

transported from the lysosome to the ER, followed by their activation to acyl-CoA catalyzed by acyl-CoA synthetases. In the ER, acyl-CoA molecules are added one by one to glycerol-3-phosphate to form TAG through the successive action of glycerol-3-phosphate acyltransferase, acylglycerol-3-phosphate acyltransferase, phosphatidic acid phosphatase, and diacylglycerol acyltransferase [122,123].

The hydrolysis of TAG in lipid droplets in cells occurs via two major and interlinked pathways. The first pathway involves the cytosolic lipases ATGL and hormone-sensitive lipase (HSL, LIPE) and likely represents the major pathway for TAG hydrolysis in macrophages. ATGL catalyzes the first step in TAG hydrolysis, producing diacylglycerol and NEFA [124], whereas HSL mainly catalyzes the second step [125]. The importance of ATGL in TAG hydrolysis in macrophages is demonstrated by the marked decrease in TAG hydrolase activity, an increase in intracellular TAG concentration, and increased lipid droplet accumulation in ATGL-deficient peritoneal macrophages, even in the absence of exogenous lipid loading [126].

The second pathway is via lipophagy, which describes the autophagic degradation of lipid droplets by LAL. Autophagy is a cellular recycling system that targets damaged or superfluous proteins and organelles, which are taken up into large double-membrane vesicles called autophagosomes and then delivered to lysosomes, where they are digested by hydrolases. While lipophagy has been shown to play an important role in the hydrolysis of cholesteryl-esters in macrophages [127], its role in TAG hydrolysis in macrophages has not been extensively addressed. Rudich and colleagues observed that oleic acid-induced lipid droplet formation was increased when the later stage of autophagy was inhibited yet suppressed if autophagy was inhibited at an early stage, leading to the suggestion that autophagy and lipid droplet formation are functionally inter-connected [128,129]. As an unrelated finding, the deficiency of ATGL and HSL in macrophages, while promoting TAG-rich lipid droplet accumulation, did not trigger a compensatory increase in lipophagy [130]. Recently, it was shown that disturbed lipophagy contributes to the intracellular accumulation of myelin-derived lipids in macrophages. Specifically, myelin-derived lipid accumulation impaired lipophagy, while stimulating autophagy reduced lipid droplet accumulation in myelin-laden macrophages [131]. Overall, more research is necessary to better understand how autophagy/lipophagy influences lipid droplet formation and degradation in macrophages and vice versa.

To understand the association between lipid storage and macrophage phenotypes, it is important to determine how macrophages make decisions on the different metabolic fates of fatty acids. Intriguingly, different types of infections, ranging from bacteria to parasites, enhance the formation of lipid droplets in macrophages [132–134], thus suggesting an obligatory link between macrophage activation and lipid storage. The storage of lipids in macrophages is essential for shaping specific functional phenotypes during various infections. Specifically, lipid droplets may actively participate in mammalian innate immunity by harboring immune proteins that have the ability to kill intracellular pathogens as well as by contributing to the local and systemic metabolic adaptation to infection [135].

Multiple lipid droplet-associated proteins have been identified that control the size of lipid droplets through the regulation of TAG storage and breakdown [16,20,121]. These include enzymes involved in TAG synthesis, enzymes involved in TAG hydrolysis, and numerous regulatory proteins. The latter group in turn includes several members of the PLIN and CIDE family, as well as ABHD5, G0S2, and HILPDA. For example, HILPDA is a hypoxia-inducible lipid droplet-associated protein that promotes lipid droplet accumulation in macrophages by inhibiting ATGL [16]. Activation of macrophages by lipopolysaccharide promotes lipid droplet accumulation by stimulating HILPDA, leading to an attenuated inflammatory response [16], thus linking intracellular ATGL-catalyzed TAG hydrolysis to the regulation of inflammatory responses. Other regulatory lipid droplet-associated proteins expressed in macrophages, such as PLIN1 and PLIN2, may also be influenced by macrophage activation and may modulate the inflammatory activation of macrophages [136,137].

Macrophages have a large capacity to store lipids, allowing them, if the fatty acid supply exceeds the energy demands, to divert the fatty acids to storage and thereby avert intracellular fatty acid accumulation and associated lipotoxicity. Besides protecting themselves from lipotoxicity, macrophages, by taking up and storing excess lipids from the environment, may also protect neighbouring cells from fatty acid overload and associated lipotoxicity.

Recently, we found that next to storing and oxidizing lipids, macrophages can also excrete part of the internalized TAG as NEFA [20]. It can be envisioned that similar to the storage of fatty acids in TAG-rich droplets, the elimination of fatty acids may serve as a mechanism to protect macrophages from lipotoxicity. By releasing fatty acids, macrophages can make the energy in TAG-rich particles available to adjacent cells, which themselves are unable to take up lipid-rich particles. Based on these considerations, the pathophysiological role of lipid-laden macrophages in certain conditions, such as atherosclerosis and obesity, might be revisited.

4. Conclusion

Macrophages come into contact with a wide variety of different lipid species and lipid particles in multiple situations in several tissues, such as the liver, adipose tissue, vascular wall, and lymph nodes. To take up the various types of lipids, macrophages use several pathways. Fatty acids can be taken up via specific transporters, including CD36 and FATPs, and most likely by endocytosis as well, although further studies are needed. LPL facilitates the uptake of LDL, VLDL, and chylomicrons by macrophages, mostly via its non-catalytic function. Similar to the uptake of fatty acids by macrophages, specific receptors, including CD36, SR-A, LDLR, and LOX-1, mediate the uptake of (modified) LDL by macrophages, followed by clathrin-dependent endocytosis. Different from LDL, macrophages take up VLDL and chylomicrons primarily by caveolae-mediated endocytosis. Interestingly, macrophages seem to have the capacity to secrete part of the fatty acids originating from the internalized TAG. In addition to the types of lipids, the size of the lipid particles impacts the route and efficacy of uptake. It is found that for the particles with the same TAG core, a smaller size leads to more efficient uptake. The lipid uptake and intracellular processing mechanisms in macrophages are summarised in Fig. 1.

It can be envisioned that the mechanisms by which lipids are taken up and processed are highly dependent on the tissue where the macrophages reside. To gain more insight into these mechanisms, more information is needed on the expressions of key proteins and receptors involved in lipid uptake across different locations and tissues. In addition, there is a need for further investigations into how specific macrophage phenotypes (classically activated, alternatively activated, metabolically activated) may differentially regulate lipid uptake and what the underlying pathways are. For example, are lipid uptake and processing mechanisms different between classically activated M1 macrophages and alternatively activated M2 macrophages, as well as other macrophage subclasses that have been identified?

Identifying the lipid uptake and processing mechanisms in different macrophage phenotypes may also reveal novel therapeutic targets to limit foam cell formation in different pathologies, such as non-alcoholic fatty liver disease, atherosclerosis, and obesity. However, when developing therapeutic strategies, it is important to consider that storage of lipids in macrophages may also serve as a protective mechanism against lipotoxicity in macrophages and surrounding cells.

Key questions of the review

1. How are different lipid-containing particles taken up by macrophages?
2. How are triacylglycerol-rich lipoprotein particles processed

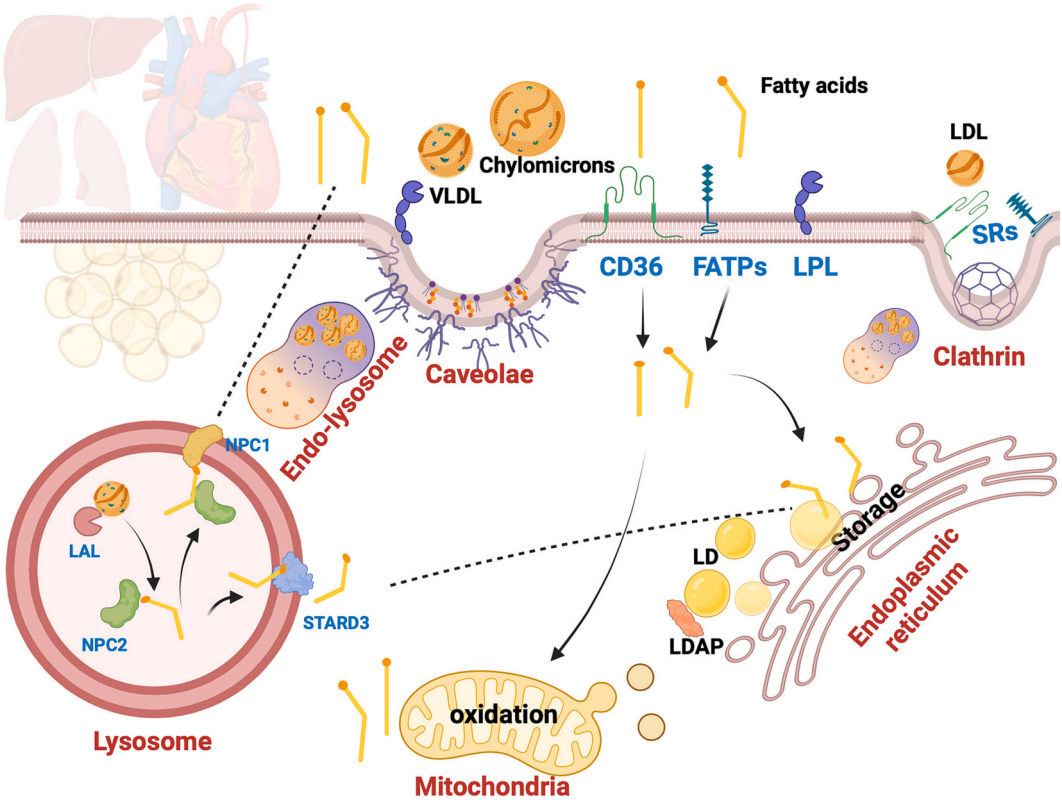

Fig. 1. An overview of the uptake and intracellular processing of different types of lipids in macrophages. At different locations, macrophages come in contact with different lipid species, including fatty acids, LDL and TAG-rich lipoproteins (VLDL and Chylomicrons). TAG-rich lipoproteins are mainly taken up via caveolae-mediated endocytosis and use the non-catalytic function of LPL. In contrast, the uptake of LDL is dependent on clathrin-mediated endocytosis, involving multiple scavenger receptors, including CD36, SR-A and LOX-1. Fatty acid uptake by macrophages is mediated by CD36 and fatty acid transport proteins, and possibly by endocytosis as well. In the endocytosis-mediated TAG-rich lipoprotein uptake process, lysosomal digestion is necessary and several important proteins are needed for lysosomal-fatty acid exportation. The fates of the intracellular fatty acids may be determined by the location or activation status of cells, which can be oxidized to provide ATP, or directed to the ER for further storage as TAG in lipid droplets (LD). Lipid droplets associated proteins (LDAP) stabilize these lipid droplets. Alternatively, macrophages can likely also excrete internalized TAG in the form of fatty acids.

intracellularly by macrophages?

3. How do physiological and pathological conditions influence the decisions regarding the storage and utilization of triacylglycerols in macrophages?

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