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Review

Review

An updated overview of lipid-regulated immunobiology in macrophages

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Abstract

Macrophages are well known for their widespread distribution, diverse roles, and involvement in multiple pathophysiological contexts, thereby constructing an immunological front line. Meanwhile, constant efforts over the past few decades have unveiled diverse reprogramming patterns of lipid metabolism as crucial, response- and context-specific drivers of macrophage functions and fate. Here, we take a bird's-eye view of major fields across the research landscape of lipid-regulated macrophages; review the latest advances in understanding how alterations in several lipid subclasses, especially their fatty acyl composition and oxidative status, direct macrophage-mediated responses and pathology outcomes; and summarize representative insights that have deciphered the lipidome composition of macrophages or profiled specific lipid species under different scenarios. We hope that this review provides readers with a handy grip to learn and explore the field of lipid-regulated immunobiology, exemplified by but not limited to macrophages.

Key words macrophage, lipid metabolism, infection and inflammation, cancer, lipid nanoparticles, fatty acids, sphingolipids, oxidized phospholipids, oxylipins, LC-MS/MS

Introduction

Initially discovered and named during the 19th century by Ellie Metchnikoff [1], macrophages are among the most prominent and intensively studied myeloid cells in the biomedical literature. They are broadly distributed across various tissues and organs, serve as sentinels to monitor molecular patterns that indicate pathogen invasion or tissue injury, and communicate with adjacent stromal cells or infiltrating leukocytes through cytokines and secretory small molecules, thereby striving to eliminate invaders, organize the extracellular matrix, or maintain tissue homeostasis [2,3]. Given their multifunctional features in different tissues, it is not surprising to observe that they exhibit context-specific heterogeneity. Distinct developmental origins and renewal routes give rise to tissue-resident and monocyte-derived macrophage populations, whereas subtle differences across tissue metabolic milieus further direct macrophages to produce unique patterns of responses [4]. Therefore, how macrophage populations react to extracellular microenvironments and reshape themselves in various ways, ultimately dictating the outcomes of myriad physiological and pathological circumstances, is an intriguing yet important question for researchers.

The metabolic network of lipids has been studied intensively in the past century, comprising a vital piece of the jigsaw puzzle in the field of biochemistry. "Lipids may be broadly defined as small hydrophobic or amphipathic molecules, partly or wholly originated by carbanion-based condensations of thioesters and/or by carbocation-based condensations of isoprene units" according to the LIPID Metabolites And Pathway Strategy (LIPID MAPS®) consortium [5]. From a functional perspective, lipids can serve as reservoirs of energy, as fundamental building blocks of cellular membranes, and as important signal carriers. From an evolutionary perspective, phospholipids assembled in diverse forms play indispensable roles in archaea, bacteria, and eukaryotes [6,7]. Metazoan species, characterized by their multicellularity, further integrate a wider array of lipids (*e.g.*, cholesterol [8]) to reinforce their membrane structure, which enables cells to withstand greater weight and tension, to assemble more complex intracellular structures, and to anchor and cluster proteins that facilitate metabolism, cell move-

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ment, and intercellular communication. Given the diversity of lipids and the ever-growing list of newly identified lipids, efforts have been made to better define lipid categories and species with comprehensibility and accuracy. To the best of our knowledge, the lipidome of mammalian cells comprises at least six major lipid categories, including fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, and prenol lipids, encompassing thousands of curated lipid species [9] that are distributed across various cellular components or extracellular spaces to execute specialized functions, many of which have not been fully elucidated.

The advance of our understanding of the mutual regulation between macrophage immunobiology and lipid metabolism is an excellent example of progress in immunometabolism. On the one hand, the response and fate of macrophages are profoundly impacted by their surrounding lipid profiles, which may contribute to negative pathological outcomes. For instance, studies on atherosclerosis pathogenesis revealed that infiltrating monocyte-derived macrophages uncontrollably engulf oxidized low-density lipoproteins (oxLDLs) produced by endothelial cells, thereby transforming into foam cells in damaged arterial walls that perpetuate the vicious cycle of atherosclerotic plaque growth, chronic inflammation, and ultimately the occurrence of fatal cardiovascular incidents [10,11]. On the other hand, macrophages can also reprogram their own lipid metabolism in response to various stimuli to regulate the type, amplitude, and duration of inflammation. This can be exemplified by the downregulation of cholesterol synthesis upon viral challenge, which reinforces interferon production for anti-viral immunity [12]. Therefore, exploration of the changes, distributions, and roles of lipids in macrophages in diverse contexts has become indispensable in the field of immunometabolism. As the field continues to expand, several critical questions remain: (i) How does the lipidome of macrophages remodel within a specific disease and tissue context? (ii) How does a certain lipidome remodeling pattern dictate specific macrophage functions? (iii) From a translational perspective, can these discoveries be leveraged to develop novel diagnostic and therapeutic approaches for different human diseases?

In this review, we begin by tracing the research focus of lipid-macrophage research over the last 65 years, highlighting both classic research fields and a few emerging studies that reveal novel insights into lipid-macrophage interplays. Next, we highlight recent progress in lipid-regulated macrophage fate and functions, with a special emphasis on fatty acyl cues in inflammation. Furthermore, we summarize studies with the macrophage lipidome profiled at different resolutions in diverse contexts, providing a comprehensive picture of the lipid composition of macrophages, together with a mass spectrometry-based perspective.

Tracing the Past and Present of Lipid-Macrophage Research

Bacterial infection and atherosclerosis pathogenesis were the two earliest scenarios in which the interplay between macrophages and lipids was revealed. In the context of pathogen insult, macrophages themselves exhibit temporally regulated production of lipid mediators with pro- (*e.g.*, prostaglandins, PGs) or anti-inflammatory (*e.g.*, lipoxins, LXs) effects to strike a balance between pathogen clearance and tissue repair [13,14]. For atherosclerosis development, the inflamed arterial intima recruits monocytes into the lesion. These recruited monocytes later differentiate into macrophages, which engulf apolipoprotein B-containing lipoproteins (ApoB-LPs), in an attempt to protect the arterial wall. However, these infiltrated macrophages take up excess LPs, forming foam cells that further contribute to the modification and retention of LPs, thereby exacerbating plaque growth [15]. Ever since these initial studies, two questions have drawn attention from both academics and industry: (i) How do lipids regulate macrophage function? (ii) Can we target lipid metabolism to treat inflammatory diseases? The efforts to answer these questions have led to our current understanding of lipid-regulated macrophage immunobiology.

In this section, we aim to provide a systematic overview of these extensive and consistent efforts. We applied bibliometric approaches to summarize the developmental traits of five fields that advanced our understanding of macrophage biology and lipid metabolism. Specifically, we searched and collected "Review Articles" with topics including "macrophage" and "lipids" from the Web of Science database, obtaining 9314 records by June 2025. We subsequently exported the titles and abstracts of these articles as research information system (RIS) documents in chronological order before VOSviewer [16] was used to extract keywords presented in each RIS document to construct bibliometric graphs (Supplementary Figure S1) that provided overviews of the frequencies, links, and clustering of keywords across different periods. Furthermore, we manually summarized the above fields and their respective high-frequency keywords from these maps to construct five comprehensive timelines (Supplementary Figure S2) that demonstrated the field-specific expansion and evolution of macrophage-lipid research across different nodules over time. Finally, to provide readers with a clear and global view, we integrated the primary focus of each field at different stages of their development into a Gantt chart (Figure 1), accompanied by visualization of their relative research popularity across nodes of time (Supplementary Figure S3). Moreover, brief introductions of lipid-macrophage interactions within each field are provided below.

Macrophage-derived lipid mediators in infection and inflammation: the tale of pro- and anti-inflammatory oxylipins

The immunobiology of macrophages is a foundational research area in which the emerging roles of diverse lipid mediators in regulating macrophage functions have been investigated. These lipid mediators share the commonalities of utilizing free polyunsaturated fatty acids (PUFAs) as substrates and going through enzyme-mediated oxidation during synthesis, which gives them the name "oxylipins". Starting in 1978, Kurland and Bockman [17] first demonstrated that monocytes and macrophages are the primary cells that produce prostaglandin E2 (PGE_2), a species of oxylipins derived from arachidonic acid (AA, C20:4), following lipopolysaccharide (LPS) stimulation. PGE_2 was later shown to increase macrophage inflammatory cytokine production (*i.e.*, interleukin-1β, IL-1β, and tumor necrosis factor-α (TNFα) [13,18] and stimulate procoagulant activities [19]. Apart from the macrophage-producing PGE_2, three other pro-inflammatory PGs, namely, PGD_2, PGI_2, and $PGF_{2\alpha}$, have also been discovered to comprise the PG family [20]. They can be produced with cell- or tissue-specificity, depending on the expression heterogeneity of several PG-synthesizing enzymes. Leukotrienes (LTs) represent another pro-inflammatory oxylipin family

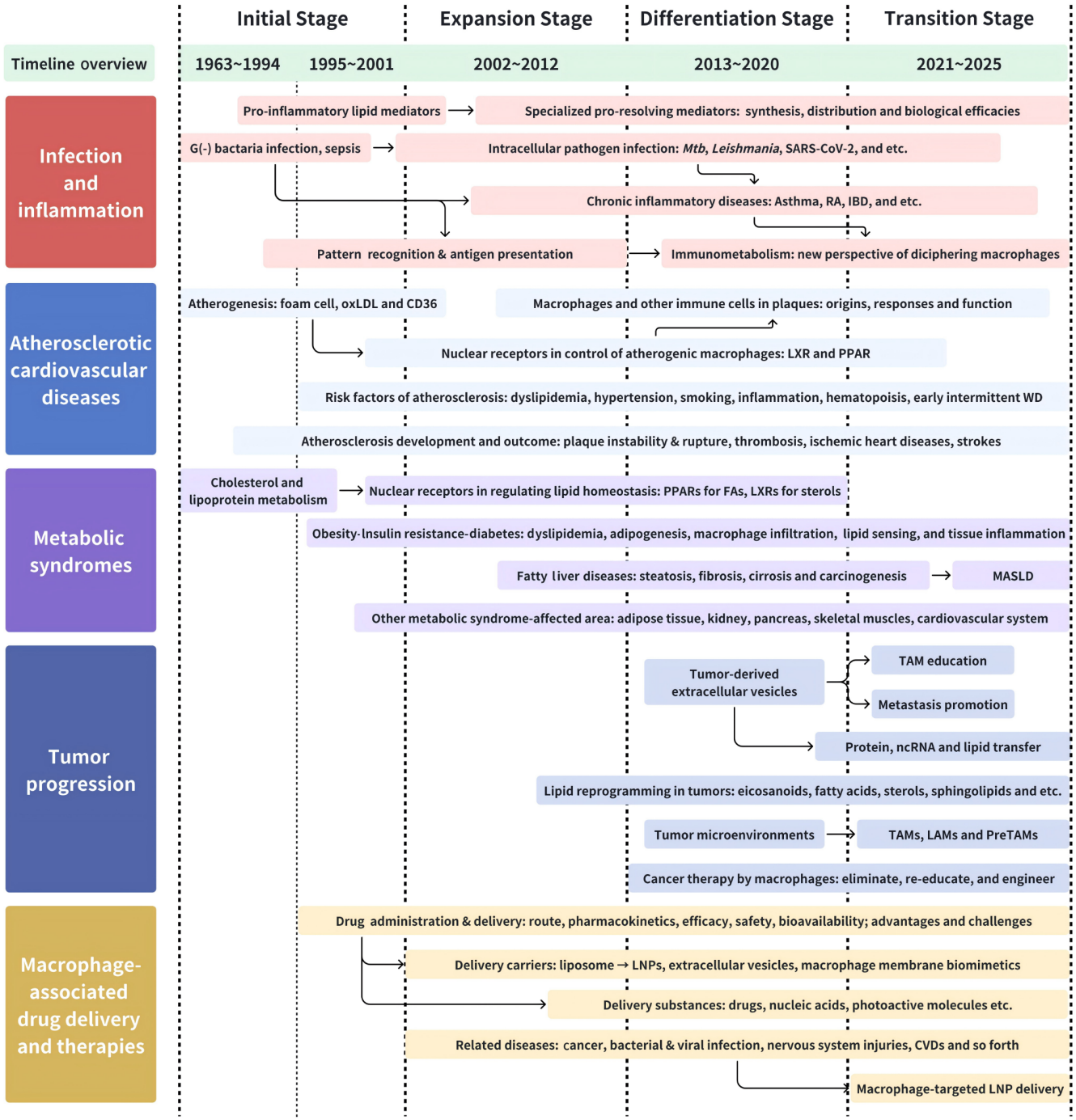


Figure 1. The Gantt chart displaying the developmental traits of five representative research fields encompassing macrophage immunobiology and lipid metabolism A timeline ranging from 1963–2025 is divided into four stages with their own durations at the top of the chart (corresponding to Supplementary Figure S3). The titles of five fields are aligned into boxes with their own theme color on the left of the chart. To their right are blocks that expand specific periods of time while displaying diverse keywords of topics within that period. Arrowheads show the hierarchical connections of topics across different blocks. Figure was created with a Feishu whiteboard. Abbreviations: G(-): gram-negative; *Mtb*: *Mycobacterium tuberculosis*; RA: rheumatoid arthritis; IBD: inflammatory bowel disease; WD: western diet; MASLD: metabolic dysfunction-associated steatotic liver disease; TAM: tumor-associated macrophage; ncRNA: non-coding RNA; LAM: lipid-associated macrophage; CVD: cardiovascular disease.

produced in various myeloid cells, including macrophages and neutrophils. Both PGs and LTs belong to the lipid class of eicosanoids (*i.e.*, oxylipins derived from C20 PUFAs). They work in concert to reduce vascular permeability and facilitate neutrophil infiltration to accelerate inflammation [21], laying the foundation for the innate immune system to limit infection and promote pathogen neutralization during the acute phase. Although these studies established the mutual regulation prototype between lipid

metabolism and macrophage-elicited inflammatory responses, little is known about how the resolution of inflammation could have been mediated by lipids at that time.

With advances in lipid profiling techniques, lipoxins (LXs) and 3 other oxylipin families were soon discovered as key lipid mediators for resolution. Infiltrating neutrophils can sense environmental PGE_2 and reprogram their eicosanoid biosynthesis pattern toward LX production [22]. As another key AA-derived oxylipin family, LXs are regarded as the first transitional signal from inflammation to resolution [23,24] by resuming vascular permeability [25], limiting TNFα-induced neutrophil diapedesis [26], and promoting non-phlogistic monocyte infiltration for macrophage differentiation [21,27]. Meanwhile, these infiltrating neutrophils gradually undergo programmed apoptosis. These cell remnants and associated microparticles, which carry abundant oxylipins and their precursors, are subsequently engulfed by tissue-resident or monocyte-derived macrophages via efferocytosis [22], inducing macrophages to synthesize pro-resolving oxylipins: resolvins (Rvs), protectins, and maresins (MaRs). These pro-resolving oxylipins further initiate tissue repair in addition to their anti-inflammatory effects [21]. Notably, although macrophages of diverse polarization stages can engulf apoptotic neutrophils, they generate polarization status-specific oxylipin patterns [22]. Overall, the above four late-onset oxylipin families, collectively referred to as specialized pro-resolving mediators (SPMs), are produced and secreted in sequence to cease neutrophil infiltration and initiate macrophage efferocytosis in a temporal order. These studies demonstrate that resolution is not merely the elimination of pro-inflammatory signals but also an active biological process that is tightly regulated in both time and space to re-establish tissue homeostasis.

In subsequent years, remarkable efforts have extended our understanding of oxylipins to more chronic contexts. For example, prostaglandins and lipoxins were shown to play opposite roles during chronic *Mycobacterium tuberculosis* (*Mtb*) infection. PGE_2 restrains *Mtb* spread by inhibiting the necrotic death of *Mtb*-infected cells to prevent the leakage of bacilli. On the other hand, LXA_4 exacerbates *Mtb* infection by increasing macrophage necrosis, which leads to bacterial release. Adoptive macrophage transfer experiments carried out with mice lacking the enzymes synthesizing PGs or LXs further supported the protective role of prostaglandins in promoting apoptosis in *Mtb*-infected macrophages, which in turn facilitates adaptive immunity against them [28]. Similarly, multiple lipid mediators exhibit distinct biological efficacy in promoting or limiting macrophage-targeted parasitic leishmaniasis [29,30]. The late-stage PGE_2/prostaglandin E receptor 2 (EP2) axis dampens lipid peroxidation-induced ferroptosis-like processes in macrophages by sustaining the activation of nuclear factor erythroid 2-like 2 (NRF2) to protect *Leishmania infantum* from clearance [30]. However, some cysteinyl-leukotriene species have been shown to exhibit *in vitro* and *in vivo* activities in diminishing *Leishmania* infection [31]. *Leishmania* species themselves are also capable of metabolizing PUFAs into precursors of eicosanoids or SPMs, which polarize macrophages into the immunosuppressive M2 phenotype, thereby facilitating their survival [29,32]. Moreover, oxylipins influence macrophage function in multiple chronic inflammatory or malignant contexts. For example, PGE_2 participates in the TNFα-dependent innate memory formation of airway macrophages in asthma [33]. The RvD_1 secreted by MER proto-oncogene, tyrosine kinase (MerTK)-positive synovial fluid macrophage subpopulations contributes to the sustained remission of rheumatoid arthritis [34]. Multiple oxylipins derived from tumor cells or tumor-associated macrophages promote tumor cell survival or impair effector T cell recruitment to facilitate cancer progression [35].

In summary, these studies support the essential perspective that lipids, particularly oxylipins, are highly regulated, functionally diverse endogenous agents produced by the host and/or pathogens to fine-tune the outcome of infection and inflammation. An imbalance in lipid mediator production or signaling is often associated with unresolved pathological circumstances. We reviewed the metabolism and immune-regulating roles of oxylipin in macrophages in more detail in section "Oxylipins in macrophage-mediated tissue repair and regeneration: from pro-inflammatory to resolution". As the list of immune-regulating oxylipins continues to grow, it is worth noting that the same oxylipin can play both pro- and anti-inflammatory roles, which are dependent on receptor expression patterns, cell types, and the inflammatory milieu. Innate immune cells have been intensively studied, as they are the main source of oxylipins and express broad repertoires of oxylipin receptors. Oxylipin receptors can also be detected on adaptive immune cells, although at a more modest level. As oxylipins can be secreted into the microenvironment and even be detected in plasma, investigating the direct effects of oxylipins on adaptive immune cells and other cell types as a mechanism for shaping the systemic immune response would be valuable. A better understanding of the cell type- and receptor-specific effects of oxylipin would allow more precise therapeutic interventions for inflammatory and infectious diseases. In addition to oxylipins, the list of other immune-regulating lipids is also expanding, exemplified by monounsaturated fatty acids (MUFAs) and ceramides, which we briefly review in section "Sphingolipid acyl chain specificity: translating metabolic diversity into immune regulation".

Atherosclerotic cardiovascular diseases: essential roles of oxidized lipids and macrophages

Atherosclerosis is defined as patchy plaques that form and develop in the intima of medium-sized and large arteries and are initiated and driven by aberrant lipid deposition and inflammation [36,37]. It is a common risk factor for stroke and ischemic heart disease, the two of which remain the top 2 causes of death in China by 2021 [38]. Intraplaque foam cells have long been observed, with macrophages being a major constituent. The mechanism of foam cell formation and how foam cell persistence influences the fate of atherosclerotic plaques have been intensively investigated.

Diverse forms of low-density lipoproteins (LDLs) play indispensable pathogenic roles in atherosclerosis development. Initial discoveries from the 1980s to 2000s revealed that injured arterial sites induce monocyte infiltration and differentiation into macrophages, accompanied by the accumulation of LDLs. These deposited LDL particles are subsequently oxidized by endothelial cells into oxLDLs, which are the primary LDL form engulfed by infiltrating monocytes, leading to foam cell formation in plaques [39]. Moreover, several types of oxidized lipids in oxLDL particles have been shown to play key roles in mediating disease progression. Notably, oxidized phosphatidylcholine (oxPC) on the oxLDL surface could be recognized by the scavenger receptor CD36, thereby bypassing the negative feedback mechanisms in the LDL-LDL receptor (LDLR) route to facilitate excess cholesterol uptake and foam cell formation [39]. The engulfment of oxLDL also imports

oxidized sterols (*e.g.*, 22(S)-hydroxycholesterol and 25-epoxycholesterol) to activate the liver-X receptor (LXR)/retinoid X receptor (RXR) complex, which triggers the expression of not only cholesterol efflux machineries but also the anti-apoptotic CD5 molecule-like (CD5L) protein, which collectively supports macrophage survival and plaque stabilization to prevent rupture [40–42]. Paradoxically, evidence suggests that knocking out *CD5L* to impair macrophage survival can reduce plaque growth in the early stages of atherogenesis [41], indicating that monocyte infiltration, survival, and oxLDL ingestion are the primary drivers of plaque growth during this period, whereas cholesterol efflux contributes less to limiting plaque size. Moreover, oxLDLs also contain oxidized unsaturated fatty acids (*e.g.*, 9- or 13-hydroxyoctadecadienoic acid (HODE)) that can stimulate peroxisome proliferator-activated receptor γ (PPARγ) to exert pro- or anti-atherogenic effects by increasing CD36 protein levels or activating LXR pathways [40]. These results indicate that monocyte-derived macrophages actively scavenge intima-deposited oxLDLs, which contain various lipid species, producing distinct regulatory effects on cells via nuclear receptor-associated pathways. However, the facts and views of atherosclerosis are renewing as our research progresses, as a recent review covering such changes questioned the participation and contribution of oxLDLs in atherogenesis in human subjects, indicating that native LDLs or LDLs aggregating with proteoglycans may provide alternative atherogenic mechanisms [43].

Lipid-induced chronic inflammation is another vital characteristic of atherosclerosis across different developmental stages, in which lipid-elicited macrophage inflammatory cascades play an indispensable role. For instance, minimal cholesterol crystals present in early atherosclerotic lesions are sufficient to damage the lysosomal membranes of macrophages, leading to NLR family pyrin domain-containing 3 (NLRP3)-inflammasome-mediated secretion of IL-1β and IL-18, promoting intraplaque inflammation [15,44]. Moreover, persistent cholesterol challenge and inflammatory stimuli in plaque-associated macrophages predispose them to apoptosis and impair their efferocytosis capacity in advanced plaques. Although the precise timing and mechanisms of efferocytosis failure remain elusive, it is postulated that reduced macrophage M2 polarization and lower production of SPMs play a part in this phenomenon [37]. On the other hand, oxLDL directly activates macrophage toll-like receptor 2 (TLR2) and promotes atherosclerotic inflammation, which involves the transition of the macrophage population, as revealed by single-cell RNA-sequencing (scRNA-seq) data. Specifically, TLR2 activation leads to the transition from the triggering receptor expressed on myeloid cells 2 high ($TREM2^{hi}$) lipid-associated macrophage (LAM) population to the perilipin 2 high ($PLIN2^{hi}$)/$TREM1^{hi}$ population, the latter of which exhibits elevated *Il1b* and C-C motif chemokine ligand 2 (*Ccl2*) transcription patterns and is associated with carotid plaque complications leading to cerebrovascular events in human cohorts [45].

Furthermore, a recent study focused on resident arterial macrophages revealed that distinct dietary patterns strongly influence certain macrophage subpopulations. Specifically, in the early stage of life, compared with continuous hyperlipidemia, western diet-induced intermittent hyperlipidemia depletes aortic-intima-resident, atheroprotective, lymphatic vessel endothelial hyaluronan receptor 1 positive ($LYVE1^{+}$) macrophages, leading to exacerbated atherosclerotic development in mid-adulthood [46]. This study highlights a novel yet essential role of arterial-resident macrophages in atherogenesis and demonstrates the detrimental effect of dysregulated lipid exposure in depriving this protective macrophage subpopulation in children and adolescents, which in turn elevates their future risk of atherosclerosis development. In summary, cholesterol, oxLDL-associated lipids, and certain patterns of diet accelerate atherosclerosis progression by remodeling not only macrophage function but also the composition of macrophage subpopulations.

To understand the discoveries and research trajectory of atherosclerosis, we can summarize its canonical pathogenic pattern into four key characteristics, *i.e.*, arterial damage-initiated, dyslipidemia-driven, macrophage-intertwined, and inflammation-exacerbated. LDL and macrophages have long been the focus of researchers studying atherosclerosis, and the emergence of specific lipids in LDL/oxLDL, as well as novel macrophage subpopulations, has largely helped us understand the progression of this life-threatening disease. As discussed in section "Macrophage-derived lipid mediators in infection and inflammation: the tale of pro- and anti-inflammatory oxylipins", the profile of oxylipins largely shapes their pro- and anti-inflammatory status. Therefore, it is intriguing to explore whether fine-tuning the lipid mediator of inflammation, such as promoting more efficient tissue repair after arterial intimal injury, resolution of arterial inflammation, and stabilization of the formed plaque, could help fine-tune atherosclerosis-associated inflammation. On the other hand, this field also highlights the value of scRNA-seq-empowered analysis of macrophage subpopulations in understanding disease progression in great detail. Given that certain macrophage subpopulations have an enhanced ability to take up lipoproteins within the plaque, can we use an oxLDL decoy as a way to precisely target this macrophage population? Finally, dietary interventions are important and cost-effective approaches for treating atherosclerosis at different stages. In addition to long-term caloric restriction (CR), other diet regimens, such as the Mediterranean diet [47], DASH [48], and MIND [49], which combine both regimens, are being rigorously tested for their ability to normalize lipid profiles and improve patient outcomes. How distinct diets reset systemic lipid metabolism and influence monocyte recruitment, infiltration, and activation needs to be further investigated, which would pave the way for precise diet intervention for distinct populations.

Metabolic syndrome-associated diseases: systemic lipid dysregulation induces macrophage-mediated inflammation

Multiple chronic metabolic diseases, such as type 2 diabetes and metabolic dysfunction-associated steatotic liver disease (MASLD, previously known as nonalcoholic fatty liver disease (NAFLD)), affect overweight and obese individuals more prevalently. Specifically, this portion of the population mainly exhibits hypertension, hyperglycemia, dyslipidemia, and too much fat around the waist, which can be collectively referred to as metabolic syndrome [50]. If left untreated, it can cause systemic stress on multiple tissues and organs, simultaneously impacting not only parenchymal cells but also stromal cells to induce degeneration.

In adipose tissue and the liver, resident and monocyte-derived macrophages in the stroma respond to symptoms of metabolic syndrome and generally exacerbate its progression. For instance, adipose tissue hyperplasia and adipocyte hypertrophy are two compensatory responses that buffer persisting overnutrition condi-

tions. The expansion of adipose tissues results in increased levels of monocyte chemoattractant protein-1 (MCP-1), which facilitates monocyte infiltration, as supported by genetically induced or diet-induced obesity in mouse models. Moreover, enlarged adipocytes release saturated fatty acids (SFAs) into the tissue microenvironment, which activate adipose tissue macrophages via TLR4 signaling to elicit the production of pro-inflammatory cytokines (*e.g.*, TNFα and IL-6) [51,52] that drive obesity-induced insulin resistance [53]. In addition to SFAs, other metabolic syndrome-associated lipids, including cholesterol crystals and oxidized phospholipids, can serve as priming or triggering signals to induce NLRP3 inflammasome assembly, caspase-1 activation, and gasdermin (GSDM) cleavage, resulting in IL-1β secretion in diverse macrophage populations (summarized in [54]) that further intensifies insulin resistance [55] and MASLD [56]. In addition, macrophages are able to sense endogenous β-glucosylceramide released by dead adipocytes via the pattern recognition receptor Mincle, forming the well-known crown-like structure (CLS) that is also similarly observed in the fibrotic liver. These CLS-forming macrophages promote stromal cell death-induced inflammation and fibrosis, thereby aggravating lesions in metabolically stressed organs [57]. These discoveries highlight the vital role of macrophages as essential sensors and conductors in metabolic syndrome-mediated diseases via lipid-pattern recognition receptor interactions and inflammation induction.

Extensive research on obesity-induced chronic metabolic diseases has focused on a similar pathogenic cycle, where lipotoxicity, insulin resistance, and macrophage-mediated chronic inflammation reciprocally exacerbate each other to promote metabolic syndrome-associated organ lesions, including adipose tissue hyperplasia, pancreatic islet inflammation [58,59] and MASLD. Dysregulated sphingolipid and glycerophospholipid metabolism has been studied in hepatocytes; however, whether these changes also apply to macrophages requires further investigation. Powered by single-cell-level techniques, precise monocyte and macrophage subpopulations contributing to disease progression are identified, and the common signature shared by these subpopulations needs to be analyzed. From a broader perspective, whether and how these dysregulated organs communicate with or influence each other during the progression of various diseases are largely unknown. A recent report in which pigs were used as model animals provided a systemic metabolite production and consumption dataset across 10 organs under scenarios of a fasting/feeding cycle, a western diet, and LDLR deficiency, along with corresponding tissue transcriptomics and hormone arrays [60]. Surprisingly, the Western diet and LDLR deficiency triggered extrahepatic organ-derived bile acid (BA) release into the circulation, which may contribute to vascular inflammation and atherosclerosis development. Since macrophages also express BA-activated receptors [61], the intricate mechanisms by which these BAs systematically modulate metabolic dysregulation or immune responses await investigation.

Cancer: lipid reprogramming between macrophages and tumor cells

Hypoxia and nutrient deficiency represent two significant characteristics of tumor microenvironments (TMEs), in stark contrast to those of metabolically overloaded tissues and organs in metabolic syndromes [62]. Such extreme conditions, which could be explained by the high energy and biomass demands of cancer cells, recruit inflammatory monocytes and train both resident and monocyte-derived macrophages to gain new features, a process that somewhat resembles tissue repair. These infiltrating macrophages within the solid tumor niche are generally defined as tumor-associated macrophages (TAMs), which are well known for their immunosuppressive and cancer-supporting roles.

TAMs tend to accumulate sterol and other lipids via direct uptake via CD36 and other lipid transporters, phagocytosis of dying cells and debris, etc. Evidence shows that TAMs rich in lipids contribute to tumor progression. TAMs of various origins accumulate lipids into lipid droplets (LDs) to support their own fatty acid oxidation activity to boost immunosuppressive phenotypes [63]. Interestingly, in multiple mouse and human carcinoma specimens, TAMs exhibit the highest plasma membrane cholesterol enrichment among all cell types within the TME, leaving infiltrating $CD8^+$ T cells deficient in plasma membrane cholesterol and dampening their tumoricidal capacity [64]. Furthermore, TAMs synthesize 25-hydroxycholesterol (25-HC), which accumulates in macrophage lysosomes to activate adenosine 5′-monophosphate-activated protein kinase (AMPK)-mediated signal transducer and activator of transcription 6 (STAT6) signaling to increase arginase 1 (ARG1) and IL-10 expression, which in turn promotes tumorigenesis [65]. In addition, a recent study provided direct evidence that TAMs take up lipids in the TME through the scavenging of myelin debris by CD36, which not only rewires their epigenetic profiles to acquire immunosuppressive features but also fuels cholesterol into glioblastoma cells to promote their proliferation and recurrence [66].

On the other hand, tumor cells also shuttle lipids into the TME, which educates TAMs, as melanoma cell-derived glucosylceramides induce unconventional endoplasmic reticulum (ER)-stress responses downstream of Mincle in TAMs to promote their survival while activating STAT3 signaling to increase the expression of immunosuppressive genes [67]. Furthermore, some studies have shown that pancreatic cancer cells produce exosomes with a high AA composition in their membrane phospholipids, which facilitates exosome-TAM fusion [68,69]. TAMs can convert engulfed AAs into PGE_2, a pro-inflammatory lipid mediator that promotes the survival of breast cancer cells [35,70]. Collectively, these results illustrate a vital “depot” role of TAMs in reshaping the distribution and metabolic profiles of lipids, especially sterols, to increase tumor cell fitness and dampen the functions of endogenous tumoricidal cells in the TME. Moreover, their immunosuppressive traits can be further enhanced by tumor cell-mediated lipid transfer to facilitate cancer progression. However, the exact routes of lipid accumulation in TAMs and their potential for targeting need to be further investigated.

Notably, scRNA-seq and lineage-tracing technologies further clarified the temporal heterogeneity of intratumor macrophages, distinguishing them into PreTAMs with a tissue-resident origin that have contributed to neoplastic transformation versus TAMs that are recruited from the peripheral blood after tumor formation [71], adding another layer of complexity in macrophage-mediated tumor onset and progression. Studying the distinct lipid profiles of PreTAM and recruited TAM populations and understanding how they contribute to tumor onset and progression is highly valuable.

Targeting macrophages with lipid-based carriers for developing precise therapeutics

From the above discussions, we can clearly observe the pivotal roles

of macrophages in different pathogenic scenarios. On the basis of these discoveries, researchers from the fields of lipid biology, immunology, oncology, and nanotechnology have focused on macrophages as valuable targets for therapy development. Among various methods, the use of lipid nanoparticles (LNPs) has received increasing attention as a safe, low-immunogenicity, highly modular *in vivo* substance delivery approach [72]. Its wide application in coronavirus disease 2019 (COVID-19) vaccines further validated the feasibility of LNPs for *in vivo* delivery of nucleic acids while avoiding unexpected immune activation.

An emerging trend in the development of macrophage-targeting LNP formulations is underway. In fact, genetic manipulation of macrophages has long been considered challenging and has largely restricted macrophage-based cell therapy. However, macrophages are excellent targets for LNP-based therapy. LNP administration via routes where macrophages are abundant can lead to preferential LNP uptake by macrophages as a natural consequence of their phagocytic clearance role. In addition to passive targeting, other strategies to enhance macrophage targeting include LNP composition optimization, antibody conjugation, and ligand decoration, which are briefly discussed below.

Currently, macrophage-targeting LNPs have been tested in various diseases, such as solid tumors, infectious diseases, and tissue fibrosis. Two studies using sigma-1 receptor-targeting LNPs (intravenous injection) [73] or mRNA-siRNA co-encapsulating LNPs (tumor *in situ* injection) [74] achieved successful delivery of their cargos into the tumor lesions of the mouse pancreas, thereby inducing TAM reprogramming, which reduced intra-tumor fibrosis [73] and improved tumor control. Moreover, chimeric antigen receptor-macrophages (CAR-Ms) have shown great potential in killing tumor cells, as dual delivery of mRNAs encoding CAR-Ms and Siglec-G lacking immunoreceptor tyrosine-based inhibition motifs (ITIMs) by LNPs (systemic injection) achieved liver macrophage targeting, generating *in situ* CAR-Ms as well as CD24-Siglec-G blockade, which promoted liver macrophage phagocytosis and tumor burden relief. In addition to their effects on cancer, macrophage-targeting LNPs have also been applied to other pathological circumstances. In influenza-associated pneumonia, F4/80-conjugated, TGF-β-activating kinase 1 (TAK1)-siRNA-loaded LNPs have been engineered to target alveolar macrophages to prevent excess inflammation [75]. CRV peptide-modified, mRNA-siRNA dual-loaded LNPs have been formulated for successful *in situ* programming of CAR-M, which efficiently engulfs and clears methicillin-resistant *Staphylococcus aureus* (MRSA) [76]. Moreover, phosphatidylserine-containing *Mms6* mRNA-loaded LNPs have been applied to target macrophages in spinal cord lesion sites to increase motor function recovery [77].

The investigations and development of novel lipids and lipid derivatives for constructing targeted LNPs constitute another research field for achieving safe and efficient delivery. More naturally, macrophage-targeting mechanisms need to be tested for distinct organs and tissue microenvironments. Moreover, small molecules or genetic manipulations targeting lipid metabolism-associated enzymes or transporters, lipid-sensing receptors, or kinases downstream of lipid perturbance have shown promising therapeutic potential in animal or preclinical models [65,78–80]. Therefore, combining mechanisms of lipid-regulated pathogenesis with novel macrophage-targeting LNPs could further unleash the therapeutic potential of RNA delivery-mediated macrophage modulation, thereby paving the way to overcome the limits of existing remedies in tackling infection, inflammation, tissue regeneration, and cancer.

In summary, the emergence and development of each field are closely interconnected, as revealed by insights into the physicochemical properties, biological activities, and metabolic patterns of different lipid species. Macrophages are undoubtedly the carriers and skilled practitioners of these insights, which endows them with broad plasticity to adapt to ever-changing circumstances and execute distinct functions. From this perspective, in addition to further revealing the reliance of macrophages on lipid metabolism, practical efforts are needed to exploit the lipid-guided functional plasticity of macrophages and transform these mechanistic insights into clinically applicable therapeutics.

Fatty Acids and Beyond: Emerging Lipid Mediators in Macrophage Function and Disease

Along the paths of discovery, characterization, and functional investigation of lipids in human diseases, fatty acids and sterols are among the earliest lipid classes that have been extensively studied owing to their prominent accumulation in scenarios of metabolic dysregulation. Subsequent research revealed their involvement in both infection and cancer progression by mediating the functions of macrophages and other immune cells. Given their essential roles in the lipid network, these two lipid classes continue to be key subjects of study, with more derivatives being identified and novel immune regulatory roles further revealing that they constitute a vital portion of immunometabolism [81–83].

Fatty acids and sterols are fundamental building blocks of almost all mammalian lipids. They can be referred to as simple lipids since they cannot be broken down by hydrolysis. The analysis of distinct fatty acids within biological samples was developed in the 1950s and 1980s, when gas chromatography (GC) and GC–MS became accessible for lipid analysis, respectively. Unlike cholesterol, which is present in its simple form in membranes, fatty acids are present as fatty acyl tails in complex membrane lipids. It was not until the early 2000s, with the introduction of the concept of "lipidomics," that we were able to identify and quantify complex lipids with their fatty acyl composition, bond type, and modification in detail in a high-throughput manner. Here, we provide a brief overview of the roles of fatty acid metabolism and several emerging lipid species in regulating macrophage function in various contexts.

Fatty acid composition is the key defining functional diversity of membrane complex lipids

The functional diversity of complex lipids emerges from four key variables: (i) fatty acid composition, including chain length, saturation degree, and branching patterns that influence membrane properties and metabolic fate; (ii) chemical bonds that link fatty acyl tails to the backbone, including mainly ester bonds but also amide, alkyl ether or vinyl ether bonds; (iii) identity of the polar headgroup, which dictates their subcellular localization, protein interactions, and signaling specificity; and (iv) a range of enzymatic and non-enzymatic modifications, such as glycosylation, oxidation, and other chemical alterations that generate bioactive derivatives with distinct regulatory functions. The biological importance of these structural complexities becomes apparent when the intricate enzymatic networks that govern lipid metabolism in immune cells are considered. As illustrated in Figure 2, the formation of diverse

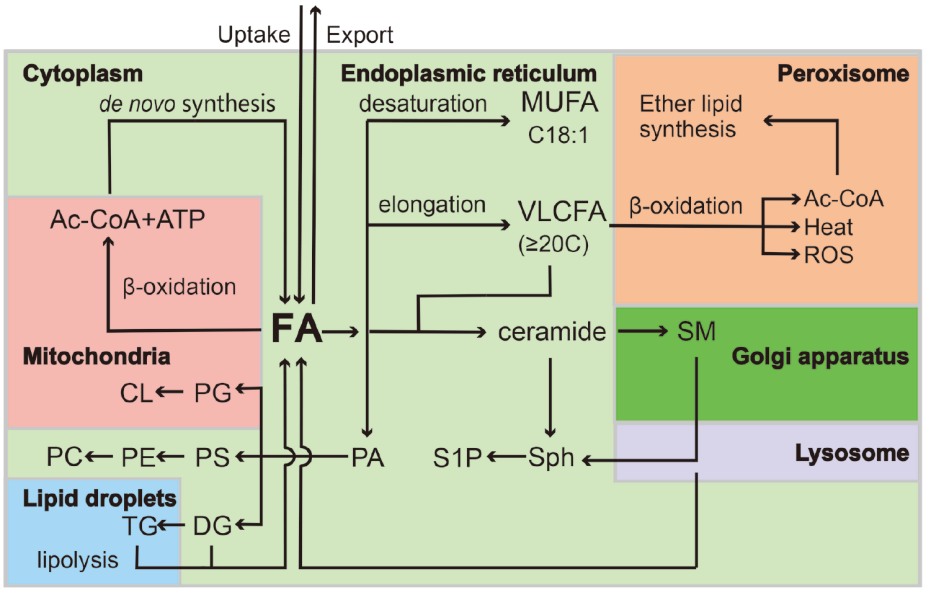


Figure 2. Subcellular organization of fatty acid metabolism and integration into complex lipids Cellular fatty acid homeostasis involves coordinated processes across multiple organelles. Fatty acid acquisition occurs through extracellular uptake, cytoplasmic *de novo* synthesis, or lipid droplet lipolysis. Metabolic fates include mitochondrial β-oxidation for energy production or cellular export. Chain modification and complex lipid integration take place in the endoplasmic reticulum (ER), where fatty acids undergo elongation and desaturation before being incorporated into complex lipids such as ceramides and phosphatidic acid (PA). Specialized peroxisomal processing handles very-long-chain fatty acids (VLCFAs) through distinct β-oxidation pathways that also promote ether lipid synthesis. Multi-organelle sphingolipid processing involves ceramide trafficking through the Golgi apparatus and lysosomes to generate diverse sphingolipid species, including sphingosine (SPh) and sphingosine-1-phosphate (S1P). Glycerolipid diversification occurs across various cellular compartments, where PAs serve as precursors for multiple glycerolipid classes that fulfill distinct functions in energy storage, signal transduction, and membrane structure. This compartmentalized organization enables precise control over lipid composition and function in response to cellular demands.

cellular lipid arrays relies on fatty acid profiles shaped by the balance of uptake, synthesis, chain modification, and degradation. Cells can obtain fatty acids via uptake or *de novo* synthesis [84]. Imported free fatty acids are activated to acyl-CoAs by long-chain acyl-CoA synthetases (ACSLs) [85] for further conversion and utilization. *De novo* synthesis is mediated by the fatty acid synthase complex (FASN) [86]. Chain modification is achieved by either elongation of the very-long-chain fatty acid protein (ELOVL) enzyme family for elongation [87] or stearoyl-CoA desaturase (SCD) [79] together with fatty acid desaturase (FADS) [88] for desaturation. The metabolic disposal of fatty acids includes mitochondrial β-oxidation, which generates ATP for energy production, and peroxisomal β-oxidation, which produces heat and handles very-long-chain substrates [89].

The incorporation of fatty acyl groups, as part of complex lipid biosynthesis, results in remarkable chain selectivity. For glycerophospholipids (GPLs), glycerol-3-phosphate acyltransferases (GPATs) and lysophospholipid acyltransferases (LPLATs) [90] incorporate specific fatty acids into the *sn*-1 and *sn*-2 positions of distinct GPL classes, respectively, whereas phospholipase A_2 (PLA_2) members [91] selectively hydrolyze the *sn*-2 acyl chain with distinct chain lengths and saturation preferences, serving as mobilizers of the GPL-attached fatty acid reservoir. For sphingolipids, serine palmitoyltransferase (SPT) initiates synthesis primarily via C16:0 acyl-CoA at the *sn*-1 position, whereas 6 distinct ceramide synthases (CERS) determine *sn*-2 acyl chain length [92–94]. Notably, CERS2 and CERS3 preferentially utilize very long-chain fatty acids (VLCFAs, FA chain length ≥ 20), whereas other CERS isoforms favor shorter acyl chains—a specificity that directly impacts sphingolipid function in immune regulation.

The advancement of mass spectrometry has led to the identification and characterization of novel lipid species with more complex structures and modifications, such as phospholipids with alkyl ether or vinyl ether bonds, sphingolipid species attached to various fatty acid chain lengths or glycosylation, and oxidatively modified lipids [65,95,96]. By combining mass spectrometry with advanced scRNA-seq or spatial sampling techniques, we are at the beginning of deciphering the production route and *in vivo* distribution of various lipids, facilitating a more thorough discovery of their functional implications in immunological or pathological contexts. As a result, accumulating evidence supports the idea that dynamic coupling between fatty acid remodeling and lipid class turnover supports macrophage function during inflammation. To illustrate how these structural features translate into immunoregulatory outcomes, we focus on three paradigmatic examples: (i) sphingolipids, which exemplify the critical role of fatty acid chain diversity in shaping immune responses; (ii) oxidized phospholipids, which demonstrate how oxidative modifications transform integral membrane lipids into novel signaling molecules, thereby creating novel signaling molecules; and (iii) oxylipins, which represent a specialized class of oxidation-derived lipid mediators that integrate metabolic and immune signaling. Together, these case studies reveal how the interplay between lipid structure and function drives the sophisticated regulatory networks underlying macrophage biology.

Sphingolipid acyl chain specificity: translating metabolic diversity into immune regulation

The chain length and saturation level of the fatty acyl group of

membrane lipids critically influence membrane fluidity [82], lipid raft formation [97,98], protein-lipid interactions [99], and lipid turnover, ultimately determining whether a lipid species promotes inflammatory activation or resolution in macrophages.

VLCFAs are the most abundant fatty acids in myelin. Chung *et al.* [100] demonstrated that during demyelination or aging, glial cells are exposed to elevated levels of VLCFAs, driving their conversion of excess VLCFAs into sphingosine-1-phosphate (S1P) via a glial-specific S1P biosynthetic pathway. Accumulated S1P triggers neuroinflammation via nuclear factor-kappa-B (NF-κB) activation and recruits macrophages into the central nervous system (CNS). Genetic suppression of S1P signaling in *Drosophila* glia or neurons or pharmacological blockade with fingolimod (an S1P receptor antagonist) strongly attenuates VLCFA-induced phenotypes. Conversely, increasing VLCFA levels in glia exacerbates neuronal dysfunction. In vertebrates, elevated VLCFA and S1P are also toxic in the experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis. Reducing VLCFA levels with bezafibrate alone or in combination with fingolimod synergistically improved EAE outcomes, suggesting dual targeting of VLCFA and S1P as a promising therapeutic strategy for multiple sclerosis.

Next, a previous work from Bensinger's group exemplifies the power of dissecting the interdependence of fatty acyl-lipid classes in immune regulation. Using shotgun lipidomics, they showed that distinct pathogen-associated molecular patterns (PAMPs) activate different TLRs and downstream signaling axes (*i.e.*, myeloid differentiation primary response protein 88 (MyD88) vs TIR domain-containing adapter-inducing interferon-β (TRIF)), leading to signal-specific lipidomic reprogramming across lipid classes [79,83]. Isotopic tracing and fatty acid synthesis analysis (FASA) further revealed that TRIF-dependent TLR activation suppresses the *de novo* synthesis of SFAs and MUFAs, with increased long-chain fatty acids (LCFAs) derived from extracellular sources. In contrast, MyD88-dependent TLR activation (*e.g.*, TLR1/2, TLR7, and TLR9) upregulates the nuclear factor erythroid 2-related factor 2 (NRF2)-sterol regulatory element binding protein 1c (SREBP1c)-SCD axis to increase SFA and MUFA synthesis. Notably, the accumulation of MUFAs during later periods of TLR activation (24–48 h) is crucial to the negative feedback regulation of MyD88-driven inflammation, although the mechanism remains unclear. York *et al.* delved deeper into this question in the context of inflammatory bowel diseases [78]. They demonstrated that SCD2-mediated MUFA accumulation in macrophages after TLR2 activation prevents aberrant sphingolipid synthesis in an IL-10 signaling-dependent manner. On the other hand, loss of IL-10 signaling drives the synthesis and accumulation of saturated VLC-ceramide species, which in turn prolong macrophage-mediated colon inflammation by facilitating the nuclear translocation of REL at later periods of TLR activation (24–48 h), intriguingly aligning with the buildup time of MUFA. These two studies collectively suggest that the remodeling of the intracellular fatty acid pool dictates the temporal compositional alterations of sphingolipid species, thereby precisely tuning the intensity and duration of macrophage inflammation.

Collectively, these studies highlight a multilayered regulatory network in which fatty acid chain dynamics, particularly the balance between MUFA, VLCFA, and their incorporation into sphingolipids, critically shape macrophage function. Rather than examining individual lipid species, we recognize that lipid functions emerge from the coordinated actions of metabolic enzymes that together determine their structural composition, lipid class specificity, and production timings. This integrated view not only deepens our understanding of immunometabolism but also helps to identify promising therapeutic targets, such as those targeting VLCFA metabolism or sphingolipid remodeling, for treating inflammatory, neurodegenerative, and metabolic diseases.

Oxidized phospholipids in immunometabolism: from atherosclerosis to infection

Oxidized phospholipids (oxPLs) represent a critical class of lipid mediators that profoundly shape macrophage polarization and function (Figure 3) [96,101–104]. Chemically, oxPLs are phospholipids whose PUFAs side chains or polar head groups have undergone oxidative modifications through free radical reactions or enzymatic processes. Here, we highlight two seminal studies from the Zanoni group that exemplify the evolving understanding of oxPL biology—from their role in sterile inflammation to their emerging functions in host defense and immunometabolic regulation.

The first study [102] revealed that oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (oxPAPC) is a complex mixture that induces a "hyperinflammatory" state in phagocytes upon LPS stimulation and during atherosclerosis development. Distinct molecular species within the oxPAPC mixture drive divergent functional outcomes: POVPC and PGPC promote inflammasome activation without triggering pyroptosis, leading to the formation of hyperactive macrophages. In contrast, PEIPC induces a "hypermetabolic" state characterized by increased IL-1β accumulation. This hyperactivation is mechanistically dependent on metabolic reprogramming, including: (i) concurrent activation of oxidative phosphorylation (OXPHOS) and aerobic glycolysis; (ii) glutamine-dependent replenishment into the TCA cycle; and (iii) cytoplasmic accumulation of oxaloacetate (OAA), which stabilizes HIF-1α and amplifies inflammatory signaling. Notably, macrophages in atherosclerotic lesions recapitulate these metabolic features, underscoring the pathophysiological relevance of oxPL-driven immunometabolic rewiring [102].

While oxPLs are well-established drivers of chronic inflammatory diseases such as atherosclerosis and metabolic dysfunction-associated steatohepatitis (MASH; previously known as nonalcoholic steatohepatitis (NASH)), their roles in various infectious contexts are more complicated. Previous studies established the pro-inflammatory capacity of oxPLs in influenza infection [105,106], as well as the intricate phenomenon of oxPL exposure in alleviating or exacerbating inflammation before or after bacterial challenge, respectively [107]. A subsequent study from the same group [96] significantly advanced our understanding of oxPL biology in infection, revealing a conserved mechanism of immunometabolic control across disease settings. PAMP stimuli generate host-derived oxPLs that rapidly accumulate in macrophages and dendritic cells *in vivo*. Although they exacerbate inflammation, they do not alter pathogen burden, suggesting a decoupling of immunopathology from microbial control. Mechanistically, oxPLs inhibit AKT signaling, rewire one-carbon metabolism via the methionine cycle, and promote genome-wide enhancer of Zeste 2 polycomb repressive complex, subunit 2 (EZH2)-mediated H3K27me3 modifications, leading to epigenetic silencing of *Il10*. Pharmacological blockade of oxPLs increases IL-10 production, reduces inflammation, and improves survival, highlighting their

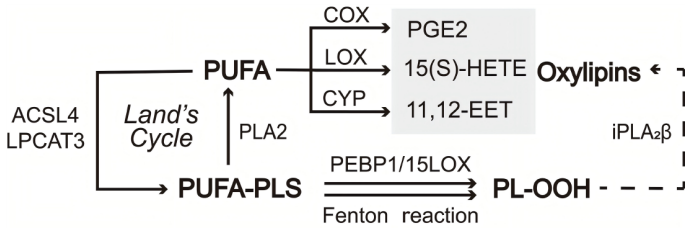


Figure 3. PUFA metabolism and oxidative lipid mediator generation: from Lands cycle to immune regulation Polyunsaturated fatty acids (PUFAs) undergo dynamic metabolic cycling and oxidative processing to generate diverse bioactive lipid mediators. Lands cycle operation involves PUFA incorporation into phospholipids (PUFA-PLs) via acyl-CoA synthetase long-chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3), followed by selective PUFA release through phospholipase A_2 (PLA_2) activity. Free PUFA oxidation occurs through multiple enzymatic pathways, including cyclooxygenases (COXs), lipoxygenases (LOXs), and cytochrome P450s (CYPs), generating a diverse array of oxylipins with distinct immunoregulatory functions ranging from pro-inflammatory to pro-resolving activities. Phospholipid peroxidation occurs through both enzymatic (PEBP1/15LOX-mediated) and non-enzymatic processes (Fenton reaction), producing oxidized phospholipids (oxPLs) that exhibit complex immunomodulatory properties. Specialized hydrolysis of peroxidized phospholipids is preferentially catalyzed by calcium-independent phospholipase $A_2\beta$ ($iPLA_2\beta$), enabling selective mobilization of oxidized fatty acid species. This integrated network of PUFA metabolism and oxidation creates a sophisticated lipid signaling system that fine-tunes immune responses across different physiological and pathological contexts.

potential as therapeutic targets. Intriguingly, lipid peroxidation plays paradoxical roles in macrophage-mediated immunity. While it can induce ferroptosis of intracellular bacteria, contributing to host defense [108], lipid peroxidation also drives gasdermin-D (GSDMD)-mediated pyroptosis in host macrophages during polymicrobial sepsis, where the antioxidant enzyme GPX4 reduces both lipid peroxidation and septic lethality [109]. This duality suggests that the functional impact of oxPLs depends critically on the nature of the pathogen, the timing of infection, and the local metabolic landscape.

These two seminal studies from Zanoni's group expanded our understanding of oxPLs in the maintenance of tissue homeostasis and revealed the impacts of oxPLs on epigenetic regulation and inflammasome modulation in macrophages. Given the multifaceted roles of oxPLs in oxidative damage, immunometabolic reprogramming, epigenetic regulation, and host defense across sterile and infectious contexts, these broader perspectives raise several compelling questions. (i) Do oxPLs represent a convergent metabolic checkpoint shared across divergent macrophage activation states [102,104]? (ii) What are their precise origins—enzymatic oxidation, ferroptosis-induced secretomes [110], or generalized oxidative stress—and how do these sources influence function? (iii) Can oxPLs serve as precursors for bioactive oxylipins in an enzyme-dependent manner, and do antioxidant systems such as GPX4 [103,109] indirectly shape immune responses by regulating oxPL metabolism? Collectively, oxPLs are not merely bystanders of oxidative damage but also dynamic signaling molecules that integrate metabolic cues with immune outcomes across disease contexts, positioning them as pivotal regulators at the interface of chronic inflammation, infection, and tissue repair.

Oxylipins in macrophage-mediated tissue repair and regeneration: from pro-inflammatory to resolution

Oxylipins are a diverse class of bioactive lipid mediators derived from the oxidation of PUFAs through the enzymatic actions of cyclooxygenases (COXs), lipoxygenases (LOXs), and cytochrome P450s (CYPs) and non-enzymatic peroxidation [111]. As reviewed in section "Macrophage-derived lipid mediators in infection and inflammation: the tale of pro- and anti-inflammatory oxylipins", while early oxylipin research focused predominantly on pro-inflammatory mediators [111], the field has undergone a paradigm shift toward understanding resolution-promoting oxylipins, which offer direct therapeutic potential for treating chronic inflammatory diseases [112–114]. Unlike classical cytokines, certain oxylipins act locally with short half-lives, enabling precise spatiotemporal control over cellular responses [115] and limiting their effects to injury sites while restricting stem cell activation to discrete regenerative windows, thereby preventing aberrant tissue remodeling. The growing appreciation of macrophages as metabolic and signaling hubs has highlighted their capacity to produce distinct oxylipin profiles depending on the context, thereby instructing neighboring cells through paracrine lipid signaling [115]. Notably, increasing evidence shows that shifts in the oxylipin profile are tightly linked to aging, fibrosis, and regenerative decline, underscoring their therapeutic relevance [95,115]. This evolving paradigm positions oxylipins at the interface of immunometabolism and regenerative biology, motivating a closer examination of how macrophage-derived oxylipins contribute to tissue repair, even in contexts traditionally associated with cell death, such as pyroptosis.

To dissect the functional contribution of metabolites and oxylipins to the pyroptotic secretome, Mehrotra *et al.* [115] employed the $Pyro^{-1}$ model, in which anthrax lethal toxin (LeTox) induces pyroptotic cell death in bone marrow-derived macrophages (BMDMs) via activation of the NLRP1B inflammasome without triggering the release of IL-1β or IL-1α. This model circumvents the dominant pro-inflammatory effects of IL-1β, which may cloud the potential roles of other bioactive molecules released during pyroptosis. PGE_2 was enriched in the Pyro-1 secretome. Its *de novo* synthesis is induced during pyroptosis through a caspase-1-COX-2 axis, and its release is dependent on GSDMD-mediated pore formation. Notably, metabolites and oxylipins present in the supernatant of the Pyro-1 model promote wound healing *in vivo*, which is correlated with increased recruitment of $CD301^+CD206^+$ $IL\text{-}27^+$ reparative macrophages to injury sites. Intriguingly, COX activity, which is responsible for PG biosynthesis, appears to be activated during the late stages of pyroptosis, suggesting tightly regulated temporal coordination of lipid mediator production.

Consistent with these findings, Chi *et al.* [95] utilized a 3-kDa cellulose membrane to remove IL-1β and other large cytokines selectively. However, they reported that the residual oxylipins and small metabolites in the pyroptotic secretome retained their tissue-reparative capacity. These data support a paradigm in which

macrophages release pro-healing oxylipins, namely, 11,12-epoxyeicosatrienoic acid (11,12-EET), in a GSDMD dependent, non-canonical manner during tissue injury. 11,12-EET, a bioactive oxylipin, is synthesized by soluble epoxide hydrolase (Ephx2) and released through GSDMD pores. It functions as a potent regenerative cue through modulating liquid-liquid phase separation (LLPS) of fibroblast growth factor (FGF) to enhance its signaling, thereby promoting the activation and proliferation of muscle stem cells and accelerating muscle regeneration. Importantly, Ephx2 expression and 11,12-EET production decrease with age, contributing to impaired muscle regeneration. Exogenous supplementation with 11,12-EET restores muscle mass and strength in aged mice, highlighting its therapeutic potential. These findings underscore the emerging role of oxylipins as key lipid mediators in regenerative medicine and identify Ephx2 and its metabolites as promising molecular targets for anti-aging interventions.

Recent studies have further elucidated how the functional output of macrophage-derived oxylipins differs across the lifespan [116]. While adult macrophages typically promote fibrotic scarring upon organ injury, neonatal macrophages are indispensable for tissue regeneration. This functional dichotomy is partly attributed to the lipid mitogens generated during efferocytosis, which support receptor-mediated regenerative programs. Neonates possess uniquely polarized tissue-resident macrophages capable of efficient efferocytosis. Genetic ablation of the MER proto-oncogene tyrosine kinase (Mertk), a key receptor for apoptotic cell recognition, completely abrogates cardiac regenerative capacity in neonates. Mechanistically, Mertk-dependent efferocytosis reprograms the metabolism of neonatal $C1q^{+}TLF^{+}$ macrophages, driving the biosynthesis of thromboxane A_2 (TXA_2). TXA_2 acts in a paracrine manner on neighboring cardiomyocytes expressing the thromboxane receptor (TP), stimulating their proliferative capacity and supporting heart regeneration. In contrast, adult macrophages exhibit suppressed TXA_2 production following efferocytosis. The short half-life of TXA_2 restricts its action to cells in close proximity to the site of production, functioning as an autocrine or selective paracrine signal. This spatial constraint prevents aberrant activation of distal cell populations and ensures precise control over regenerative signaling. Together, this efferocytosis-TXA_2 axis exemplifies the central role of macrophages as signaling hubs in tissue repair, with the physicochemical properties of oxylipins—rapid synthesis, localized action, and short duration-enabling tight spatiotemporal regulation while also highlighting the developmental plasticity of macrophage-derived oxylipin metabolism across different life stages.

Collectively, these findings extend the conceptual and clinical landscape of oxylipins as fast-response, *in situ*-produced lipid mediators that promote tissue repair beyond their traditional roles in inflammation [111]. Mechanistically, they not only function as ligands [117] but also modulate membrane dynamics and phase separation to influence receptor clustering and signaling. Their dual capacity to modulate immune responses and to instruct parenchymal cell behavior puts them at the intersection of immunometabolism and regenerative biology. Notably, given that tumors are often described as “wounds that do not heal”, it is conceivable that pro-regenerative oxylipin pathways may be co-opted in the tumor microenvironment to support immune evasion, stromal remodeling, and cancer cell proliferation [118,119]. Moreover, these insights prompt a re-evaluation of widely used drugs such as non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit COX enzymes and may inadvertently suppress beneficial oxylipin production during tissue repair [120,121]. Future therapeutic strategies may aim to selectively preserve or enhance reparative oxylipin pathways while dampening pathological inflammation, offering new avenues for treating aging-related degeneration, chronic wounds, and metabolic disorders.

Profiling the Lipid Atlas of Macrophages: A Contemporary Understanding

As illustrated in the previous sections, the characterization of detailed fatty acyl compositions in complex lipids and their interconnecting metabolic dynamics with fatty acids is crucial for revealing insights into how macrophages maintain physiological homeostasis or promote disease pathology. High-quality lipid characterization relies fundamentally on two key steps: the precise separation of individual lipid species from complex biological mixtures, followed by detailed molecular fingerprinting. This critical combination has driven the widespread adoption of liquid chromatography (LC) coupled with mass spectrometry (MS) for comprehensive lipid profiling [122]. Notably, the advancement of soft ionization techniques in the late 1980s enabled the gentle charging of neutral molecules, paving the way for the use of MS in complex lipid profiling. Currently, electrospray ionization (ESI)-based high-performance liquid chromatography-tandem mass spectrometry (HPLC-ESI-MS/MS) has become the mainstream technique for lipidomics profiling. Moreover, its combination with trapped ion-mobility spectrometry (TIMS), electron-activated dissociation (EAD), and other modules increases the separation efficiency and resolving accuracy of lipid molecules (*e.g.*, the application of EAD facilitates the characterization of detailed double-bond positions of each fatty acyl connected to different *sn*-positions in glycerolipids), enabling us to uncover numerous macrophage immuno-metabolic insights into different pathophysiological scenarios [81]. Similarly, researchers have also utilized macrophages as a model to develop new lipid analytical methods and create standardized databases encompassing diverse cell origins or stimulation conditions, thereby generating a valuable repository of lipid dynamics for further study and exploitation.

Accordingly, we review key studies that (i) depict macrophage lipidome compositions from various angles and (ii) establish experimental pipelines for accurately detecting specific lipid classes or species. Moreover, we provide two tables (Tables 1 and 2) featuring complementary information on the discussed articles within the two respective topics, aiming to help readers grasp the essential purposes of each article, as well as their similarities and differences from multiple perspectives.

Mapping lipidomes in macrophages: the ever-growing territory

Given the identification of major lipid metabolic enzymes and pathways, scientists have begun to wonder how the cellular lipidome changes under various biological circumstances. Since macrophages play vital pathophysiological roles in infection, inflammation, and homeostasis maintenance, they have been chosen as the ideal model cell type to monitor lipid alterations under these scenarios. The mouse RAW264.7 cell line has been the most widely used macrophage cell type for lipidome profiling and for the development of analytical methods for common or rare lipid

Table 1. Macrophage lipidomes profiled from various sources and conditions

Published year	Cell type	Profiled lipid categories or classes	Stimuli, treatments, or scenarios	Unique perspectives	Ref.
2010	RAW264.7	FAs, Eicosanoids, GPL, GL, ST, CE, SP, CoQ, Dolichols	KLA	Time-course stimulation, 0 ~ 24h	[9]
2010	RAW264.7	GPL, CL, SP, ST, CoQ, Dolichol, ether-GPLs	KLA	Subcellular fractionation extraction 24h post stimulation	[123]
2017	RAW264.7	GL, Cholesterol, CE, GPL, Lyso-GPL, SP	LPS	Dose-specific lipidomics changes of LPS and rosiglitazone	[124]
2018	mBMDM	GL, CE, SP, GPL, ether-GPL	Laurate, myristate, palmitate, stearate (C12:0 ~ C18:0), and oleate (C18:1)	Single or dual-supplementation of Fas; Saturation degree analysis of PC, PE, and their ether-linked form	[125]
2020	mBMDM	CE, SP, FA, Lyso-GPL, GPL, ether-GPL, GL	TLR1/2, 3, 4, 7, and 9 ligands; acetyl-LDL supplement	Linked lipidome changes with TLR-immune pathway specificities	[79]
2025	Mouse microglia	Free FA, SM, TG, DG, CE, Cer, Glycosyl-Cer, PC, PI, Acyl Carnitine	APP-KI mouse Alzheimer's disease model	Aβ-stressed mouse brain-derived microglia lipidomics analysis	[126]
2016	hMDM	PC, PE, PI, PS, SM, Phosphatidylglycerol, Eicosanoids	M1 (IFNγ+LPS), M2a (IL-4) and M2c (IL-10)	Membrane lipid-centered, with detailed FA compositions at different *sn*-positions of GPLs	[127]
2024	Human or mouse immune cells	GPL, ether-GPL, Lyso-GPL, SP, Cholesterol	Naïve state, lymphocyte activation, myelopoiesis	*In vivo* immune cell lipidomics	[128]
2024	THP-1, hMDM	GPL, ether-GPL, GL, SP	*In vitro* foam cell differentiation	Single cell, multi-omics analysis including sub-population clustering & pseudo-time trajectory analysis	[129]
2024	Human intra-plaque macrophages	TG, PC, PE, Lyso-PC, alkyl ether-PC, SM, CE	Human carotid atherosclerosis	The first MSI report of lipid changes across compartments of patient plaques with or without TIA	[130]
2024	Human & mouse alveolar macrophages	Free FA, PC, PE, Lyso-PC, Lyso-PE, CE, SM, TG, DG, Cer, Glycosyl-Cer	Pulmonary alveolar proteinosis (PAP)	Temporal lipid changes during PAP treatment in patients; cross-comparison with mouse PAP models	[131]

mBMDM: mouse bone marrow-derived macrophage; hMDM: human monocyte-derived macrophage; FA: fatty acid; GPL: glycerophospholipids; GL: glycerolipids; ST: sterol lipids; CE: cholesterol esters; SP: sphingolipids; CoQ: coenzyme Q; CL: cardiolipin; SM: sphingomyelin; TG: triglyceride; DG: diglyceride; Cer: ceramide; PC: phosphatidylcholine; PI: phosphatidylinositol; PE: phosphatidylethanolamine; PS: phosphatidylserine; KLA: Kdo2-lipid A; IFN: interferon; LPS: lipopolysaccharide; TLR: toll-like receptors; IL: interleukin; LDL: low-density lipoprotein; Aβ: amyloid β-protein; MSI: mass-spectrometry imaging; TIA: transient ischemic attack.

classes and species. In particular, members of the LIPID MAPS® consortium have been the pioneering force in the field of macrophage lipidomics studies. Dennis EA and colleagues published two articles in 2010, both of which stimulated the RAW264.7 cell line with Kdo-2 lipid A (KLA, an LPS mimic), giving rise to the initial time-course (0 ~ 24 h) lipidome map [9], as well as the first subcellular organelle lipidome map in naïve and stimulated macrophages [123]. To date, LIPID MAPS® has integrated several time-course lipidomics and microarray analysis datasets of the RAW264.7 cell line, thioglycolate-elicited mouse peritoneal macrophages, and mouse bone marrow-derived macrophages on its official website, providing on-site normalized lipid species abundance comparisons and open-access lipidomics datasets across macrophage cell types and time points [142,143].

The efforts of the LIPID MAPS® consortium established the paradigm and data source for macrophage lipidomics research, serving as milestones for those who followed to blaze new trails extending farther into the world of the lipidome. Some have used ultrahigh-performance liquid chromatography (UHPLC) to achieve better lipid separation and identification efficacy [124], whereas others have switched to primary macrophages or expanded stimulation panels to mimic pathophysiological conditions [125,127,144]. Notably, Hsieh *et al.* [79] expanded the macrophage lipidome resource by generating a comprehensive lipidome map of mouse BMDMs stimulated with a full panel of TLR ligands, revealing TLR stimulus-specific lipidome reprogramming patterns and corresponding FA metabolism preferences (reviewed in section "Fatty Acids and Beyond: Emerging Lipid Mediators in Macrophage Function and Disease") that support the role of self-regulated lipidome reprogramming in fine-tuning the intensity of macrophage inflammatory responses.

Recently, the combination of lipidomics with single-cell profiling techniques, as well as flow cytometry-guided cell population sorting, has enabled the profiling of immune cell lipidomes in greater detail and from *in vivo* origins. For instance, Wang *et al.* [129] successfully developed an in-house single-cell metabolomics and lipidomics profiling and analysis workflow, which characterized two sub-populations of late-stage foam cells with distinct profiles of PUFA-containing ether phospholipid species. This result suggests a different susceptibility to ferroptosis within the two populations, which was later confirmed via single-cell transcriptomics. Another report, accomplished by Morgan *et al.* [128],

Table 2. Specialized lipid class profiling methodologies based on macrophages

Published year	Cell type	Profiled lipid classes	Stimulation or Treatment	Instruments applied	Ref.
2009	RAW264.7	Sa, So, Cer, HexCer, LacCer, GlcCer, GalCer, SM	Unstimulated	LC-ESI-QqQ and QTrap	[132]
2010	RAW264.7, mouse PM, BMDM	Ether-linked PA, PC, PE, PI, PS and PT	Unstimulated	LC-ESI-QTrap	[133]
2011	RAW264.7	eicosanoid & N-acylethanolamines	Unstimulated	LC-ESI-QTrap	[134]
2015	RAW264.7, BMDM	PAF and lyso-PAF, various species detected by untargeted lipidomics	M1 & M2 polarization, silencing of AGMO and GCH1	LC-ESI-QqQ for targeted lipidomics; LC-ESI-ionKey/MS for untargeted lipidomics	[135]
2018	RAW264.7	Complex lipid-embedded eicosanoid and oxylipins	KLA primed, ATP stimulated	LC-ESI-QTrap	[136]
2021	RAW264.7	PA from subcellular fractions	Unstimulated	LC-ESI-QTrap	[137]
2023	RAW264.7	FAs with different double-bond numbers and positions	Inhibitors of different acyl-CoA desaturases	LC-ESI-timsTOF	[138]
2024	RAW264.7	PG species, AA, 2-AG, and DG	KLA stimulation	LC-ESI-QTrap	[139]
2024	RAW264.7	PC, PC-P, PC-O, LPC, PE, PE-P, LPE, PS, PI, phosphatidylglycerol, SM, Cer, TG, DG, CE, CAR	Unstimulated or KLA stimulation	LC-ESI-Q-Orbitrap	[91]
2017	Mouse PM	Biogenic OxPC, OxPE, OxPI	Unstimulated	LC-ESI-QTOF and QqQ	[140]
2024	Primary human macrophages	hydroxyl FAs with diverse stereoisomerism	Unstimulated or IL-4	LC-ESI-QTrap coupled with multiple heart-cutting achiral-chiral LC	[141]

Sa: sphinganine; So: sphingosine, Cer: ceramide; HexCer: hexosylceramide; LacCer: lactosylceramide; GlcCer: glucosylceramide; GalCer: galactosylceramide; PA: phosphatidic acid; PT: phosphatidylthreonine; PAF: platelet-activating factor; OxPC/OxPE/OxPI: oxidized PC/PE/PI; PG: prostaglandin; AA: arachidonic acid; 2-AG: 2-arachidonoylglycerol; DG: diglyceride; PC-P/PE-P: vinyl-ether PC/PE; PC-O: alkyl ether-PC; LPC/LPE: Lyso-PC/PE; ATP: adenosine 5′-triphosphate; timsTOF: trapped ion mobility-time-of-flight; PM: peritoneal macrophage

systematically profiled the lipidomes of *in vivo* immune cells at different activation or maturation stages, generating a lipid atlas database available for online access. As a result, they described a successive decrease in PUFA-containing phospholipid (PUFA-PL) content during myeloid cell development, especially in neutrophils and monocytes. This low PUFA-PL content was subsequently shown to be one of the key factors enabling these myeloid cells to acquire ferroptosis resistance. Moreover, Wu *et al.* [126] isolated microglia from the brains of WT and amyloid beta precursor protein knock-in (APP-KI) mice for lipidomic profiling. The results revealed that lipid droplet formation and neutral lipid accumulation in microglia were further facilitated in APP-KI mice. Abrogating lipid droplet formation via macrophage-specific fat storage-inducing transmembrane protein (FIT2) knockout in microglia restored their phagocytosis and efferocytosis capacity, which in turn reduced the Aβ load in the brains of APP-KI mice. Finally, two exploratory studies collected patient-derived samples of atherosclerosis and pulmonary alveolar proteinosis (PAP), both of which are strongly associated with aberrant lipid deposition in macrophages. Using mass spectrometry imaging, the first study revealed an increase in PC and a decrease in triglyceride (TG) and cholesterol ester (CE) levels in macrophage-rich areas of plaques derived from transient ischemic attack (TIA)-positive patients compared with those derived from negative patients. Such changes in intraplaque macrophage lipid composition may indicate an elevated level of free cholesterol in the plaque, contributing to its instability and making it prone to rupture [130]. The second study provided a comprehensive yet heterogeneous alveolar macrophage lipidome landscape across three mouse PAP models and human subjects at different PAP stages, with a common and well-known trend of PC accumulation during PAP progression. The authors suggested that more efforts are needed to obtain a quantitative measurement of PAP severity from the perspective of lipids [131].

In brief, the research paradigm of *in vitro* macrophage lipidomics has reached a preliminary mature stage, with many reports and datasets available for inspection and analysis. In addition, emerging studies have made remarkable progress in extending the scope of macrophage lipidomics toward the single-cell or *in vivo* level, revealing novel alterations in lipids in various pathophysiological contexts. Notably, human patient-derived macrophage lipidomic results are emerging, offering valuable resources that warrant further investigation, provided that the necessary data collection and normalization prerequisites are met.

Advances in profiling the macrophage lipidome: methodologies and holistic views

Efforts in lipidomic profiling of macrophages have provided a holistic view of the dynamics of lipid metabolism. On the other hand, a constant stream of progress has been made in developing and implementing specialized lipid detection and analytic methodologies in macrophages. First, Shaner *et al.* [132] established multiple sets of targeted sphingolipid class quantification workflows analyzed by two types of tandem MS instruments (triple quadrupole, QqQ vs. quadrupole-linear ion trap, QTrap) in the naïve RAW264.7 cell line, and the results revealed that the QTrap can achieve greater sensitivity, albeit producing different modes of fragmentation for some of the sphingolipid compounds. Similar reports have also utilized LC-ESI-QTrap to develop profiling methodologies for various lipids, including oxidized or ether-linked glycerophospholipid species [133,140], eicosanoids, and N-acy-

lethanolamines [134,136], from RAW264.7 cells or other types of cells. In addition, the combination of an untargeted LC-ESI-quadrupole-time-of-flight (QTOF) has been proven feasible for the initial construction of a first-hand MS/MS spectral library, which can subsequently be applied to a broad-targeted LC-ESI-QqQ for monitoring endogenous, 12/15-lipoxygenase (LOX)-dependent oxidized phospholipids across *in vitro* or *in vivo* biological samples [140]. In addition, the introduction of multiple heart-cutting techniques (*i.e.*, to isolate specific portions from the first chromatographic elute into other separation conditions) into LC-MS/MS systems further enhanced the resolution of unknown lipid substances, as exemplified by Kampschulte *et al.* for coupling a second chiral chromatography with the LC-ESI-QTrap system to quantify and calculate enantiomeric fractions of hydroxy-eicosatetraenoic acids (HETEs), thereby deducing their formation routes in human macrophages [141].

These technical advances in LC-MS have promoted the discovery of subtle regulatory mechanisms in the metabolic processes of specific lipids. For example, Aleem *et al.* [139] depicted a sequential buildup pattern of PGs and PG-glycerols (PG-Gs) in KLA-stimulated RAW264.7 cells via two lipid extraction and profiling workflows. They further revealed that the *sn*-1 hydrolysis of arachidonoyl-linked diacylglycerols (DGs) to 2-arachidonoylglycerol (2-AG) and subsequent prostaglandin-endoperoxide synthase 2 (PTGS2, also known as COX-2)-mediated oxidation are the primary routes for PG-G production. Notably, it remains unclear whether the clear sequential order of PG and PG-G accumulation is related to the temporal regulatory roles of PG-G itself in macrophage functions. Watschinger *et al.* [135] revealed alkylglycerol monooxygenase (AGMO) as an IL-4-responsive lipid regulator in the RAW264.7 cell line, as AGMO silencing not only increased the levels of four alkylglycerol species but also surprisingly decreased glycosylated ceramides and cardiolipins for unresolved reasons. Moreover, they also conducted untargeted lipidomics accompanied by a panel of lipid enrichment analyses, which demonstrated wider reprogramming effects across diverse lipid categories and classes upon modulation of either AGMO activity or upstream tetrahydrobiopterin synthesis. Xia *et al.* [138] systematically outlined the enzymatic dependency of desaturation in odd-chain and even-chain MUFAs, as well as PUFAs with different chain lengths and double-bond positions, in the KLA-stimulated RAW264.7 cell line, providing a reliable resource for investigating cellular FA desaturation dynamics. Furthermore, Murawska *et al.* [91] probed the substrate specificities of three types of PLA_2 and identified AA (C20:4) in phosphatidylinositol (PI), docosahexaenoic acid (DHA, C22:6) in phosphatidylglycerol (PG), and linoleic acid (LA, C18:2) in phosphatidylcholine (PC) as the most specific *sn*-2 PUFA-PL substrates for Group IV A cytosolic PLA_2, Group V secreted PLA_2, and Group VI secreted a calcium-independent PLA_2, respectively. Although more or less traditional as these articles may seem, they have neatly combined lipid profiling with biochemical approaches to demonstrate novel metabolic mechanisms of lipids in immune cells, thereby laying the foundations for understanding complex pathophysiological scenarios associated with lipids.

In summary, the lipid profiling paradigm is shifting from measuring specific lipid classes within *in vitro* bulk cell populations toward simultaneous capture of lipid features from mixed cell populations *in vivo*, potentially coupled with transcriptional and even spatial information. As the origin, size, and complexity of lipidomics datasets continue to grow, the challenging goal remains for us to efficiently integrate and transform the increasing differences in datasets into novel biological insights through the combination of knowledge and skills from biochemistry and molecular biology with artificial intelligence, computer science, and other fields.

Concluding Remarks

In this review, we summarize the history, recent research discoveries, and technical progress related to the interface between lipid remodeling and macrophage biology. We first took a bibliometric approach to obtain an overview of the historical findings revealed across different fields. In the fields of atherosclerosis and bacterial infection, research on lipid-macrophage co-regulation has been extended to other metabolically dysregulated diseases, cancer, and targeted substance delivery over the last two decades. Through these discoveries, we can clearly see that lipids not only provide structural and energetic support but also serve as key immune signals to shape macrophage fate and function, as well as the overall innate-adaptive immune network. Next, we summarize recent findings on lipid mediators in macrophage biology, highlighting progress in understanding fatty acyl composition-mediated and oxidation-mediated immune responses. We provide an overview of a panel of enzymes or transporters located at crossroads during the uptake, biogenesis, modification, and degradation pathways of fatty acyls and complex lipids in detail, followed by some of the latest advances regarding how modifications of fatty acyls in sphingolipids, oxidized phospholipids, and oxylipins contribute to their novel regulatory roles in macrophage-mediated inflammation, infection immunity, and tissue regeneration. In the last section, we integrate progress and methods in profiling the macrophage lipidome and deciphering the features of various lipid-catalyzing enzymes, providing a practical guide for those willing to explore the subtleties of lipid metabolism in macrophages. Overall, we aim to provide a comprehensive overview of the past, present, and future of lipid research in macrophage biology, emphasizing the increasing importance of macrophages as central regulators of human physiology and disease, as well as promising therapeutic targets.

The metabolic state of a cell largely reflects its particular strategy for distributing biomass and channeling energy, which lays the foundation for how the cell senses and responds to intrinsic and extrinsic cues. Since lipids serve as both energy reservoirs and material building blocks of a cell, a thorough examination of alterations in lipid type, abundance, and modification is crucial for understanding the key metabolic features, as well as the intracellular organellar characteristics, that drive their functional externalization. This rule applies not only to macrophages but also to other immune cells, which constitute the central rationale of immunometabolism. On the basis of the wide variety of lipid species and immune cells, more insights into lipid-guided immune responses and functions are expected to be obtained in the future. Notably, we can take advantage of the macrophage-based lipid atlas and profiling techniques to study less characterized immune cells and related pathophysiological contexts.

Finally, the development of single-cell-based methods has demonstrated their strength and importance in expanding our knowledge of physiology and disease in great detail. However, its synergistic application with lipid profiling techniques for *in vivo*

samples is limited. Although technically challenging, we anticipate that combining authentic lipid features with corresponding transcriptional information at the single-cell level will enable a thorough understanding of the metabolic features of extensive macrophage subpopulations, thereby facilitating the development of more precise macrophage-targeted therapies. Moreover, we also call for rigorous attempts to transform macrophage lipid metabolism-associated mechanistic insights into clinically applicable therapies.

Supplementary Data

Supplementary data is available at *Acta Biochimica et Biophysica Sinica* online.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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