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Etiology of Lipid-laden Macrophages and in the Lung

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Abstract

Uniquely positioned as sentinel cells constantly exposed to the environment, pulmonary macrophages are vital for the maintenance of the lung lining. These cells are responsible for the clearance of xenobiotics, pathogen detection and clearance, and homeostatic functions such as surfactant recycling. Among the spectrum of phenotypes that may be expressed by macrophages in the lung, the pulmonary lipid-laden phenotype is less commonly studied in comparison to its circulatory counterpart, the atherosclerotic lesion-associated foam cell, or the acutely activated inflammatory macrophage. Herein, we propose that lipid-laden macrophage formation in the lung is governed by lipid acquisition, storage, metabolism, and export processes. The cellular balance of these four processes is critical to the maintenance of homeostasis and the prevention of aberrant signaling that may contribute to lung pathologies. This review aims to examine mechanisms and signaling pathways that are involved in lipid-laden macrophage formation and the potential consequences of this phenotype in the lung.

1.0 Introduction

Pulmonary macrophages play a critical role in innate immunity through pathogen detection, foreign substance clearance, and maintenance of surfactant homeostasis[1,2]. Due to its constant exposure to the environment, the lung is uniquely susceptible to external influence, which can alter macrophage function. The lung lining is inherently lipid rich, however, it can become hyperlipidemic in response to acute injury. As macrophages are constantly taking up lipid through phagocytosis of cells and catabolism of surfactant, this hyperlipidemia can promote the formation of lipid-laden cells. Lipid-laden macrophages, commonly referred to as “foam cells” have been implicated in the early development of atherosclerotic lesions[3,4], leading to their extensive study within the vasculature. However, macrophage lipid accumulation has the potential to play a role in other organ systems, such as the lung, where there is a growing appreciation of a role for cholesterol and lipoproteins in

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homeostasis and signaling[5], and lipid-laden macrophages have also been observed in various models of lung injury[6,7]. The presence of these large, activated cells during injury and their subsequent reduction with treatment indicate that they may play a role in the promotion of lung injury[6]. Despite these observations, it is still unclear how lipid-laden macrophages in the lung are formed or how their presence affects the inflammatory process. The purpose of this review is to examine potential mechanisms involved in lipid-laden macrophage formation and to postulate the outcomes of this phenotype in the lung.

2.0 Principles governing lipid-laden macrophage formation

Lipid accumulation in circulating macrophages in the vasculature results in foam cell formation[4,8–10], which is attributed to the uptake of damaged, oxidized, acetylated or otherwise-modified low-density lipoproteins (LDL)[8,11]. Lipoprotein uptake is facilitated through a step-wise process: receptor-mediated uptake, lysosomal processing, esterification, and storage in droplets[4], all of which if dysregulated or saturated, could contribute to excess lipid accumulation in macrophages. Mechanisms to combat excess accumulation include lipolysis and subsequent cholesterol efflux from the cell, primarily mediated through the ATP-binding cassette (ABC) transporters ABCA1 and ABCG1[3,12,13]. When intracellular cholesterol levels accumulate beyond the cellular capacity for handling and efflux mechanisms become overwhelmed, free cholesterol is esterified to form cholesterol esters (CE) which are stored as lipid droplets in the cytoplasm[4,11]. Excess CE/lipid accumulation results in the formation of lipid droplets and transforms the macrophage into a foam cell[4]. These processes can be summarized by consideration of four basic mechanisms that, when aberrant, can lead to lipid accumulation: acquisition, storage, export, and metabolism. Critically, intracellular metabolism can operate as a regulator of these processes[14]. Major proteins involved in each of these four mechanisms are summarized in Figure 1.

Pulmonary macrophages, in particular resident alveolar macrophages, exist in a lipid-rich environment due to the presence of lung surfactants[15–17]. These cells are critical to maintaining lung lipid homeostasis by constantly removing oxidized and damaged lipids, such that surfactant functions normally. The high partial pressure of oxygen in the lung and high lipid content favors lipid peroxidation and ROS generation; this leads to a disproportionate quantity of modified and damaged LDL. Lung injury has the potential to increase the quantity of lipids, especially damaged lipids, within the lung lining, which increases the load on macrophages to clear these species[18–20]. Both excessive lipid storage, uptake of oxidized lipids, and free cholesterol can result in apoptosis of macrophages, adding to the burden on the remaining phagocytic cells in the environment. Excessive oxidation and cell death contributes to injury through the release of cytotoxic mediators such as damage associated molecular-patterns (DAMPs), including heat shock protein 72[21] and high mobility group box 1 protein (HMGB1)[22], which upregulate downstream inflammatory pathways such as NF-κB, stimulate the production of reactive oxygen and nitrogen species (RONS), and recruit inflammatory cells[11,23]. Both oxidized lipids and free cholesterol can initiate the pro-inflammatory signaling cascade, however, macrophages can also esterify cholesterol, which can transform the cell into a lipid-laden macrophage and potentiate persistence and pro-fibrotic signaling[24]. In the following section, we will describe the

mechanisms by which macrophages handle lipids and how dysregulation of these processes contributes to lipid-laden macrophage formation, lung injury, and inflammation.

3.0 Cellular processes governing lipid accumulation and function of pulmonary macrophages.

3.1 Acquisition of Lipids

The physicochemical properties of lipids, which are determined by their composition and size, determine the ability of receptors to bind and transport them into the cell. Indeed, whether a particular class of lipid is taken up by scavenger receptors or endocytosed is dependent on these properties[25–27]. Anionic lipids are taken up more efficiently than their neutral counterparts[28–30], with larger diameter also increasing uptake by macrophages[31]. The lipids of the lung lining, including phosphatidylserine, phosphatidylglycerol, and phosphatidylcholine, are negatively charged, making them prone to uptake by alveolar macrophages[32–34]. Thus, one can see that the environment surrounding the alveolar macrophage will bias these cells towards lipid uptake and accumulation under physiological conditions. However, modified lipid species that may rapidly increase in the context of injury, such as oxidized cholesterol products like 7-ketocholesterol and hydroxycholesterol, common oxysterols,[35–37] have been shown to influence macrophage signaling and apoptosis. High concentrations of oxysterols affect cholesterol homeostasis and induce cytotoxicity[37,38], contributing to injury and inflammation in the lung through activation of pro-inflammatory signaling pathways, such as NF-κB[39]. This indicates that while lipid uptake and incorporation of lipid species into comparatively more inert intracellular lipid droplets promote cell survival and prolonged signaling, lack of sufficient lipid droplet formation leads to cell death and inflammatory signaling.

3.1.1 Receptor-Mediated Lipid Uptake—The scavenger receptor family of cell surface proteins are inherently vital to macrophage function recognizing a wide range of motifs that include lipids[40]. As opposed to the classical and singular uptake pathway of LDL by the LDLR[41], modified sterols, such as oxidized LDL (oxLDL) and acetylated LDL, are taken up through a variety of scavenger receptors, which can recognize both native and modified lipids[14,40,42]. Receptors known to regulate lipid uptake include Scavenger receptor A (SR-A) I/II[43,44], cluster of differentiation 36 (CD36)[44–46], scavenger receptor B (SR-B) I[44,47], cluster of differentiation factor 68 (CD68)[47], chemokine (C-X-C motif) ligand 16 (CXCL16)[47], lectin-like oxidized low-density-lipoprotein receptor-1 (LOX-1)[48,49], and cleavage factor polyribonucleotide kinase subunit 1 (CL-P1)[47]. All of these receptors have been reported to bind oxidized sterols, with the majority of oxLDL internalization attributed to SR-A and CD36[50,51]. Uptake through receptors aside from LDLR may circumvent negative feedback signaling via sterol regulatory element binding protein-2 (SREBP-2)[52], leading to excess LDL accumulation and increased cholesterol efflux. As such, macrophage scavenger receptor expression significantly contributes to the lipid-laden phenotype.

CD36 is widely expressed on macrophages and binds various modified or native proteins with a particular affinity for oxLDL[45,46]. It has been found to account for most of the CE accumulation in macrophages exposed to oxLDL[46], implicating this receptor as

one of particular interest in lipid-laden macrophage formation. Further, CD36 expression is not downregulated by the presence of intracellular cholesterol,[53] and thus lack of feedback regulation may point to this mechanism as a significant contributor to lipid-laden macrophage formation. Upregulation of CD36 has been found to be mediated through PPAR-γ activation in the presence of oxLDL, increasing its expression through a positive feedback loop[54,55]. Increased expression of uptake transporters without a concomitant increase in efflux of lipid-associated material can contribute to the development of a lipid-laden phenotype in macrophages.

3.2 Lipid Synthesis

The endomembrane, comprised of the endoplasmic reticulum (ER) and the Golgi apparatus, constitutes the primary site of lipid synthesis in the cell[56] and produces high levels of phospholipids[57,58]. The ER is directly affected by cellular stress, as can occur during lung injury, and this can impact lipid accumulation. Intracellular accumulation of free cholesterol initiates the ER stress response in macrophages[59,60], impacting a host of cellular mechanisms, including direct modification of lipid synthesis. Furthermore, macrophage functional responses are critically tied to lipid homeostasis, as the fundamental phagocytic response of macrophages requires an increase in membrane lipid production to repair cell damage and to envelop foreign matter for phagocytosis[61,62]. Below we will discuss several factors involved in lipid synthesis and its regulation.

3.2.1 Fatty Acid Synthase and Acetyl CoA Carboxylase—In the cytoplasm, Acetyl CoA Carboxylase 1 (ACC1) converts acetyl-CoA to malonyl-CoA, and in the ER, fatty acid synthase (FAS converts acetyl-CoA and malonate to malonyl-CoA[63]. In addition, the acyltransferase family of enzymes is involved in the *de novo* synthesis of membrane lipids. However, more commonly they mediate the modification of acyl chains to generate different classes of lipids[56,64,65]. Enzymes located in the mitochondria and peroxisomes also play a role in overall lipogenesis in the cell; however, the ER/Golgi and acyltransferases remain the primary drivers.

Cytosolic ACC1 regulates *de novo* FAS-mediated FA synthesis. However, in contrast to other reports of FAS-mediated lipid signaling, the ACC1-FAS pathway of lipogenesis is not critical to the macrophage mediated innate immune defenses against *Mycobacterium tuberculosis* infection[66]. Furthermore, regulation of innate immunity through lipid-mediated mechanisms may be pathogen or toxicant-specific, which is consistent with the idea that lipid content can alter chemokine response[14]. Within resident liver macrophages, inhibition of ACCs using a phosphorylation mimic results in a switch from pro-inflammatory to anti-inflammatory phenotype[67]. This suggests that the inhibition of lipogenesis may be associated with pro-inflammatory signaling.

3.2.2 SREBP Signaling—Sterol regulatory element binding proteins (SREBPs) SREBP1 and 2 are critical transcription factors for fatty acid and sterol biosynthesis[68–70]. SREBP-1a is abundantly expressed in macrophages and has been established as one link between lipid homeostasis and innate immune response[62,71,72]. SREBP1 accumulates upon acute proand anti-inflammatory activation of macrophages, and the absence of the

protein is associated with decreased fatty acid synthesis[62,73]. For example, SREBP1 is transcriptionally regulated by the pro-inflammatory Toll-like receptor 4 (TLR-4)-nuclear factor kappa B (NF-κB) pathway in macrophages[14,74]. During lung injury, saturated fatty acid levels increase and act as endogenous TLR-4 ligands to activate SREBP1 expression and subsequent lipogenesis[75,76]. Increased lipogenesis within macrophages is permissive of inflammatory activation. However, IL-4 stimulation leads to increased AKT phosphorylation, STAT6 signaling, and SREBP1-mediated upregulation of fatty acid synthesis[77], which leads to alternative activation[14]. In this regard, SREBP1 enhancement is required for alternative macrophage activation, whereas SREBP2, is not required. Thus, it is possible that SREBP1 potentiation during inflammation may contribute to eventual lipid-laden macrophage formation.

3.2.3 Mechanistic/mammalian target of rapamycin (mTOR)—mTOR complex 1 (mTORC1) is involved in lipid metabolism, proliferation, and growth, as its activation occurs in times of nutrient abundance and the absence of cellular stress[78]. The main regulatory element of mTORC1 is mTOR itself. mTOR activates SREBP1, and via mTORC1 controls the expression of binding proteins to promote lipogenesis and storage. Its inhibition has been shown to increase LDL levels in the circulation by diminishing cholesterol clearance[68]. Furthermore, a loss of mTORC function reduces macrophage mediated scavenging of LDL and increases efflux, which results in a decrease in lipid storage systemically[69,79]. The importance of mTOR activation within lung macrophages appears to be important in metabolic shifts required for activation and signaling in cancer models[73,80,81], both of which are critical to metabolic functions in lipid-laden macrophages. Despite the clear capacity of mTOR to play a role in lipid regulation, its importance in lipid-laden macrophage formation within the lung has not been delineated. This may be critical within resident alveolar macrophages, which exist in a lipid rich environment and thus can be considered to be in a state of nutrient abundance.

4.0 Lipid export in macrophages

4.1 Lysosomal Processing for Export

Prior to export, lipids are processed in lysosome-related organelles known as lamellar bodies. Lipids are broken down in the acidic interior compartment, which contains acid phosphatase, cathepsin C, and cathepsin H[82,83]. Lipolysis transforms lipids into free cholesterol and fatty acids through the action of lysosomal acid lipase[84]. Free fatty acids can then feed into the FAO metabolic pathway and subsequent mitochondrial metabolism or be incorporated as triglycerides into lipid droplets for storage within the cell. In contrast, released free cholesterol must be effluxed and chaperoned by lipoproteins, such as high-density lipoprotein or APOA1[85] or esterified in the cell to avoid pro-inflammatory signaling, cytotoxicity, and cell death[85,86] (Figure 2).

4.2 Transporter-mediated efflux

ABC Transporters A1 and G1 are the predominant cholesterol efflux transporters in macrophages, known to promote the flow of intracellular stores to extracellular carriers such as ApoA-1 or HDL[87]. The expression of these transporters is controlled by the

PPAR-γ/LXR axis[88,89]. Activation of PPAR-γ in macrophages has been shown to stimulate ABCA1 and ABCG1-dependent efflux[88,90]. Antagonists to these transporters promote lipid-laden cell formation, while activators of the PPAR-γ/LXR pathways upregulate efflux, potentially limiting the formation of lipid-laden cells.

These processes can be opposed by other signaling mechanisms that maintain lipid homeostasis in the cell. For example, the nuclear transcription factor LXR is crucial for lipid homeostatic signaling in macrophages[91,92]. Oxysterols ingested by macrophages during phagocytosis lead to increased cholesterol and oxysterol loading, inducing LXR signaling and increased transcription of ABCA1, ABCG1[91] and the inducible degrader of the LDL receptor (IDOL)[93]. Transcription of these genes oppose lipid accumulation by increasing cholesterol efflux mechanisms and degradation of the LDLR through ubiquitin-mediated mechanisms. Although the relevance of the LDLR pathway within the lung lining is unknown.

4.3 Mechanisms contributing to decreased lipid export.

As the major efflux transporters, the ABC proteins function to promote cholesterol export and mitigate lipid accumulation in the cell[87]. Therefore, dysfunction or reduced expression of these transporters can promote lipid accumulation in macrophages. Other factors contributing to reduced cholesterol efflux include depletion of cholesterol carrier proteins[94], such as apolipoprotein A-1[95], but it is unclear if this mechanism is of consequence in the lung.

Gene silencing by non-coding RNAs has been implicated in lipid homeostasis. miR-33 has been observed to decrease ABCA1 expression while knockdown of miR-33 increases reverse cholesterol transport in macrophage foam cells[96]. miR-33 targets the 3'- untranslated region of many genes involved in cholesterol homeostasis, including ABCA1[97–99]. The miRNA suppression of ABCA1 and other cholesterol efflux transports could contribute to increases in macrophage lipid accumulation.

5.0 Lipid Metabolism in Macrophages

5.1 Lipid Recycling by Macrophages

Within the lung, macrophages are continuously exposed to a lipid-rich environment, regardless of injury. They play a significant role in surfactant homeostasis by recycling the lipid. Pulmonary surfactant is comprised of primarily phospholipids and neutral lipids, the most common of the latter being cholesterol[17,100]. Alveolar type II (AT2) and Clara cells are also highly involved in lipid metabolism, producing lipids critical to surface active function[101]. If not recycled by AT2 cells, surfactant is degraded by alveolar macrophages[16,101]. Alveolar macrophages are thus needed to clear lipids even under normal physiological conditions.

5.2 Fatty acid oxidation (FAO)

Fatty acids in the cytosol are enzymatically converted to fatty acid acyl-CoA. Further downstream oxidation and energy release occurs within the mitochondria. Carnitine

conjugation and transport via carnitine palmitoyl transferase 1 (CPT1) facilitates the movement of the fatty acid acyl-CoA into the mitochondria, where carnitine is removed by carnitine palmitoyl transferase 2 (CPT2)[102]. Oxidation of these molecules yields the reducing equivalents NADH and $FADH_2$ as well as acetyl-CoA, which is used to generate energy throughout the citric acid cycle and the electron transport chain. ACC2, which is located in the mitochondrial membrane, regulates FAO by controlling fatty acid uptake to the mitochondria via CPT1[103].

Reliance on FAO is associated with enhanced cellular lifespan and limits lipid and fatty acid accumulation in macrophages[102]. In contrast, *in vivo* work has shown that constitutive activation of the transferase responsible for long-chain fatty acid import to the mitochondria, CPT1, reduces lipid accumulation in the cell[105]. Furthermore, FAO is induced by STAT6 and PPAR-γ-co-activator 1β[84,106] which are anti-inflammatory mediators, and CPT2 deletion was shown to impair FAO[105,107], oxidative phosphorylation and anti-inflammatory activation. Collectively, these data indicate a critical role for FAO in regulating lipid accumulation and macrophage phenotype. Products of lipid metabolism, such as free fatty acids, triglycerides, diacylglycerides, and ceramides, as well as oxidized lipids, influence pro-inflammatory signaling[23]. High concentrations of oxysterols affect cholesterol homeostasis and can induce cytotoxicity[37,38], contributing to injury and inflammation in the lung.

6.0 Lipid Storage in Macrophages

6.1 Lipid droplet formation

Triglycerides and cholesterol esters are the primary components of lipid droplets, encapsulated by a phospholipid membrane, and thus the accumulation of all of these components is critical to lipid droplet formation[108]. Esterification is a critical step in mitigating free cholesterol-induced cytotoxicity. The Acyl-coenzyme A cholesterol acyltransferase/sterol O acyl transferase (ACAT/*Soat*) subset of enzymes catalyzes cholesterol esterification in macrophages, with two primary isoforms identified as ACAT-1/*Soat1* and ACAT-2/*Soat2*[109–111]. ACAT-2/*Soat2* is localized to hepatocytes and the intestine, whereas ACAT-1/*Soat1* is expressed primarily in macrophages and is the principal mechanism in lipid droplet formation in this cell type[65,112,113]. ACAT-1/*Soat1* converts cholesterol and oleoyl coenzyme A to esterified cholesterol with coenzyme A as a by-product, contributing to lipid droplet formation[109–111]. The cholesterol esterification rate can be modified via extracellular signaling, as ACAT-1/*Soat1* expression can be induced through the leptin-JAK/PI3K pathway[114] and the insulin-Erk/JNK pathway[115,116]. Esterification of cholesterol by ACAT-1/*Soat1* is opposed by the action of neutral cholesterol ester hydrolase (nCEH), which releases free cholesterol for export from the cell[117].

6.1.1. Triglycerides in lipid droplet formation and turnover—In addition to esterified products, triglycerides can form the core of intracellular lipid droplets. ER-associated transferase enzymes acyl-CoA:diacylglycerol acyl transferase-1 (DGAT-1) and acylCoA:diacylglycerol acyl transferase-2 (DGAT-2) participate in triglyceride synthesis from diacylglycerol and acyl-CoA derived from FAs[118]. Conversely, triglycerides are

liberated back to diacylglycerol and acyl-CoA through various lipases found in the cytoplasm[119]. Triglycerides may also be broken down within macrophages through lipase activity in the autophagosome[120]. Thus, the dynamic balance between triglyceride formation and breakdown is a key regulator of lipid droplet formation in macrophages.

6.2 Lipid droplet signaling

Lipid droplets, comprised of the neutral lipids triacylglycerol and CE[108,121], have enzymes localized on their surface that regulate various pathways such as triacylglycerol synthesis and rates of their own accumulation and degradation[122,123]. Because lipid droplets store many different lipid types, their release can result in them directly acting as or being converted to signaling molecules[121,122]. The breakdown of triacylglycerol and CE results in free fatty acid formation, which can bind many cell surface and intracellular receptors that are stimulators of inflammatory signaling[122,124–126]. Notably, free fatty acids are able to bind and activate intra- or extracellular G-protein coupled receptors (GCPRs), toll-like receptors (TLRs), PPARs, and NF-κB, all of which are highly involved in macrophage regulation, signaling, and phenotype, potentially contributing to the pathophysiological conditions present in the lung during injury[122,124–126].

Additionally, lipid droplet formation may also impact cell signaling by removing substrates necessary for bioactive, pro-inflammatory lipid signaling. By acting as a repository for fatty acids, cholesterol, and other species, lipid droplet formation reduces the capacity for the formation of lipid peroxidation, preventing pro-inflammatory signaling[122,127]. Though this may help to prevent or lessen oxidative damage in the lung, the excessive accumulation of lipids within macrophages will alter lipid droplet metabolism and the function of lipid droplet-associated proteins[122,128]. As such, it is proposed that accumulation of lipid droplets in macrophages bias the cell towards reliance on FAO and oxidative phosphorylation rather than glycolysis. This has the potential to negatively affect macrophage function, as macrophages will persist, taking on a pro-resolution phenotype rather than undergoing homeostatic turnover and cell death which could lead to long-term signaling effects such as fibrosis. Similar dysfunction has been observed in dendritic cells, where lipid droplets prevent proper antigen presentation and chaperone-mediated autophagy[128–131], drastically altering cell function through fundamental metabolic mechanisms.

7.0 Conclusions

As reviewed here, lipid-laden macrophage formation has been observed in a variety of pathophysiologies, such as atherosclerosis, but knowledge is lacking as to their role in lung pathology. Recently, there has been considerable interest in the pulmonary macrophage and its heterogeneous roles in lung injury[1]. In this review, we have focused on the mechanisms that can lead to the formation of lipid-laden macrophages, or “foam cells”, as these cells seem to have their own unique roles. It appears that these cells may limit acute activation and bias the injury response to repair, as well as significantly altering phagocytosis and surfactant recycling[1]. When considering lipid-laden macrophage formation, there are four main processes to consider: lipid import, metabolism, storage, and export. The balance between these processes is critical to maintaining normal macrophage function under

homeostatic conditions. However, these four processes are influenced and altered by signaling, genetic factors, and – vital in the case of injury – the tissue microenvironment[132]. These processes and their regulation are summarized in Figure 2. In this way the formation of a lipid-laden macrophage can be considered as being dependent upon the balance between acquisition and synthesis with export and metabolism.

Distinct from other tissue environments in which foam cells have been characterized, the pulmonary environment is heavily lipid laden at normal physiological levels, as approximately 90% of the lung lining fluid is comprised of phospholipid[133]. In addition to their role in innate immunity, macrophages contribute significantly to the regulation of lung function and work of breathing through the catabolism of surfactant. With this role in the consistent turnover of lipid species, it is significant that pulmonary macrophages do not become inherently lipid laden under homeostatic conditions. It is proposed that the accumulation of lipid-laden macrophages in the lung is significant in lung pathophysiology, especially in the context of lung injury and inflammation. This phenotypic change may reduce the capacity of the lung to sufficiently resolve injury. The accumulation of lipids in macrophages has the potential to prolong the life of the cell[134], likely due to the reliance on FAO and anti-inflammatory phenotypic switching. These cells may be persistent and contribute to chronic signaling, promoting the transition to fibrosis rather than injury resolution[24]. Increased cellular persistence, aberrant signaling, and maintenance of a pro-fibrotic phenotype stand out as the most significant potential consequences of lipid-laden cell formation in the context of lung injury. Thus, limiting the formation of lipid-laden macrophages in lung pathologies may be an advantageous proposition from a translational standpoint. Though any of the four processes may be a feasible target for pharmacological development, we propose that targeting the excess lipid storage may be the most viable and least disruptive to normal macrophage processes. With a limited storage capacity, macrophages exposed to excess lipids would become susceptible to cell death via the cytotoxic effects of sterols[84]. Limiting excess lipid accumulation and restoring macrophage homeostasis in the context of injury may also have significant effects on the phenotypic differentiation of both resident and recruited cells.

The formation and persistence of lipid-laden macrophages in the lung have the capacity to impact the spectrum of inflammation and resolution in virtually all pulmonary pathologies. For example, the prevalence of lipid-laden macrophages may prime these cells towards the use of FAO due to substrate availability rather than glycolysis, which may ultimately impair macrophage function in various pathologies (Fig 3). More research is needed to assess how inhibition of lipid accumulation in the lung specifically may represent a pharmacological target in lung injury and disease. Additionally, there are many outstanding questions regarding the formation, prevalence, composition, and significance of this phenotype in experimental animal models and in human pulmonary insult and disease. It would be most pertinent to document the occurrence or lack of lipid-laden macrophages in lung pathologies, as well as interrogate the mechanism leading to their formation, as it may be that regulating key proteins in lipid and cholesterol handling are differential within and among lung pathologies.

From the various gatekeepers of lipid-laden macrophages presented within, there are many potential therapeutic targets to prevent the formation of this cell phenotype in the lung. It is of note that many of these, such as limiting scavenger receptor expression and activity[135] have been explored in the context of other disease states like atherosclerosis. However, altering receptor uptake in pulmonary macrophages inherently blunts their phagocytic capacity, pointing to this strategy as ineffectual in this organ-specific context. Activation of the LXR signaling pathway leads to the inhibition of enzymes directly catalyzing lipid droplet storage such as the DGAT or ACAT enzymes. This may present as a logical target to inhibit lipid-laden cell formation, however, macrophage-specific targeting of LXR in the lung remains challenging. The use of mi-RNA knockdown, such as with the investigation of miR27 for the prevention of atherosclerosis[136] may eventually useful. Ultimately, there are inherent risks in immunomodulation as well as in the practical application of the strategies, limiting the discussion of viable therapeutic targets at this time. More detailed research into the formation of pulmonary-specific lipid-laden macrophages is necessary to understand their role in lung pathologies.

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Highlights

- Lipid-laden macrophages are observed in the lung *in vivo* following pulmonary injury, and this phenotype may significantly contribute to pulmonary pathologies.
- This phenotype is proposed to form due to the imbalance between lipid acquisition, metabolism, storage, and export.
- The complex interplay between metabolism and storage of lipids are discussed as they pertain to disease progression.
- Herein, we discuss critical regulators and signaling pathways that may play a role in the formation of lipid-laden macrophages in the lung.
- The consequences of lipid-laden macrophage formation within the context of pulmonary inflammation are considered.

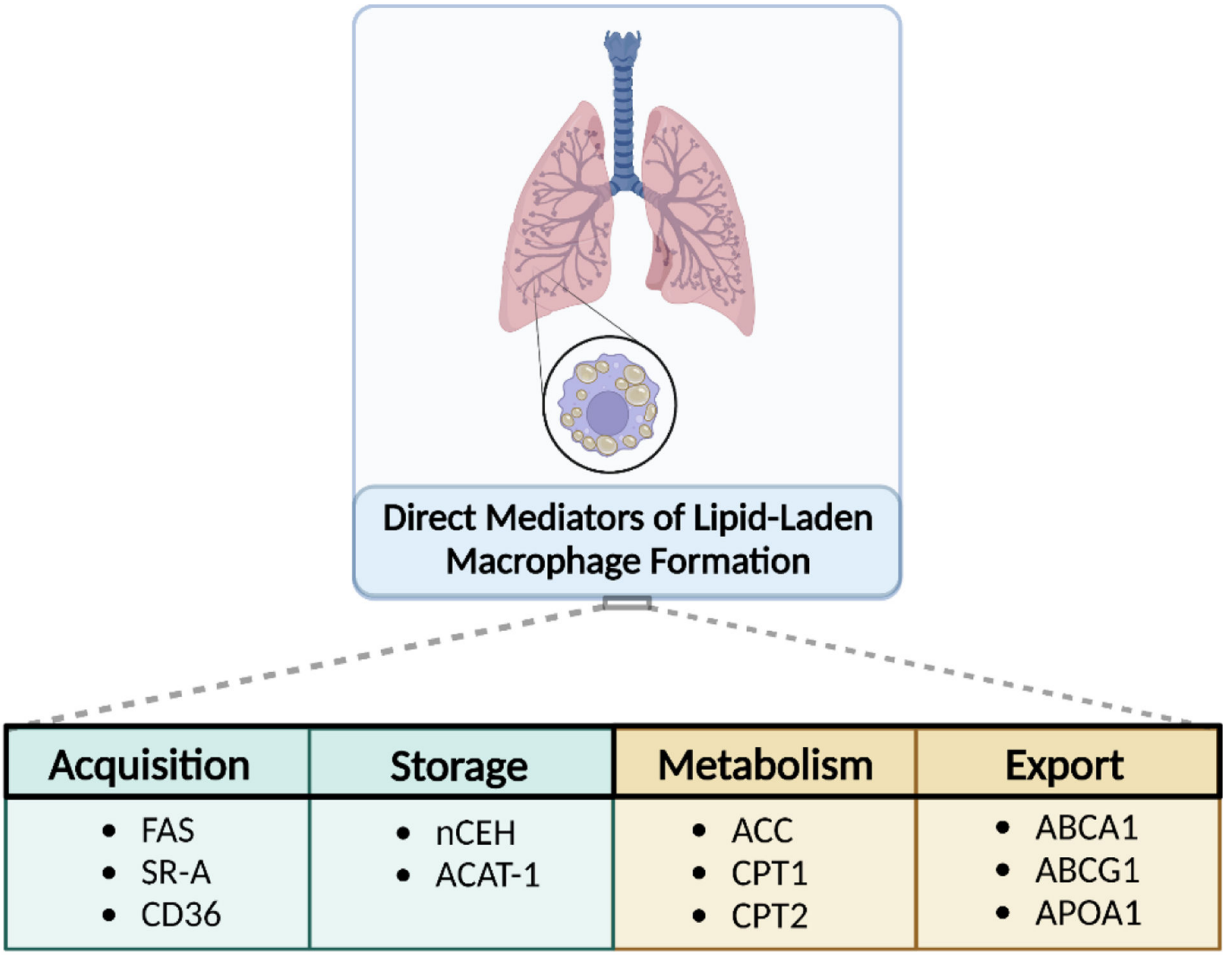

Figure 1.

Balance between lipid acquisition, export, metabolism, and storage in pulmonary macrophages are the primary processes that prevent the development of a lipid-laden phenotype. Dysregulation or imbalance of these processes leading to the excess storage of lipid are the most significant when considering the development of this cell phenotype and potential therapeutic intervention in the lung.

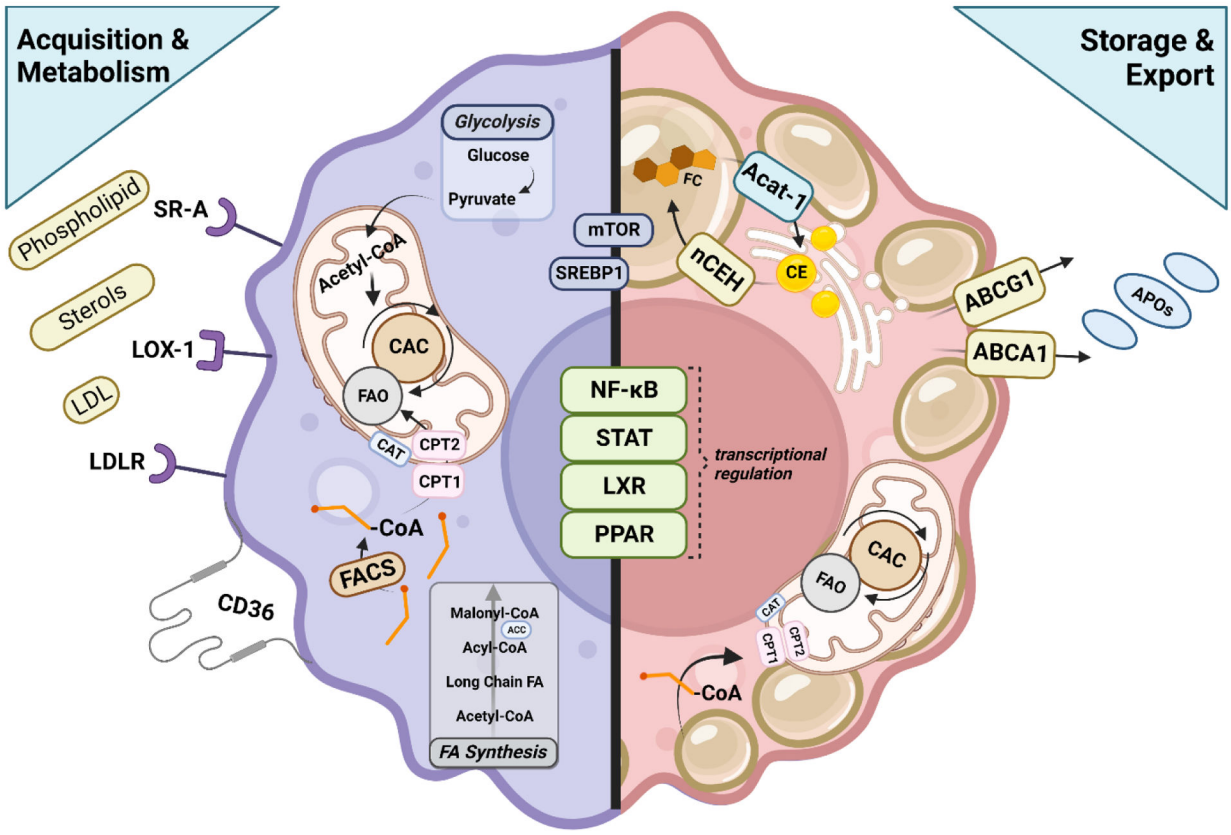

Figure 2. Cellular processes governing lipid accumulation in alveolar macrophages.

Macrophages acquire lipids and cholesterol through a variety of receptors including SR-A, LOX-1, LDLR and CD36. These cells also acquire lipid through *de novo* fatty acid synthesis. FACS conjugates free Co-A to the molecule, allowing for the conversion of the fatty acyl CoA to acyl carnitine, which can then be transported across the mitochondria membrane by CAT. CPT2 converts acyl carnitine back to fatty acyl CoA which can then be oxidized and feed into the CAC. NADH, $FADH_2$, and GTP produced from glycolysis/CAC are oxidized and the resulting electrons flow through the complexes of the electron transport chain, creating a protein gradient that drives ATP synthase to produce usable energy for the cell in the form of ATP. Upregulated storage of lipids in the macrophage drive sterol-sensitive signaling pathways, some of which oppose lipid accumulation. LXR signaling induces the transcription of cholesterol efflux transporters like ABCG1 and ABCA1 to promote lipid homeostasis within the cell, similar to PPARα-mediated reduction in triglyceride levels. PPARγ signaling increases glucose metabolism and upregulates the expression of CD36, promoting the uptake of lipids in the macrophage and, along with STAT6, also induces FAO. Contributing to increased substrate availability for FAO, NFκB controls the transcription of pro-inflammatory genes and contributes to macrophage signaling, including activation of SREBP1 which promotes lipogenesis, a process also controlled by mTOR signaling. Importantly, the cell must also have mechanisms to store lipid to be oxidized at a later time for energy and to counteract free cholesterol-induced cytotoxicity, thus storage of these molecules is vitally important. Free cholesterol is esterified by ACAT-1, forming CE, the critical reaction leading to lipid droplet formation

in the macrophage. This action is opposed by nCEH which releases free cholesterol for export for loading onto various apolipoprotein carriers.

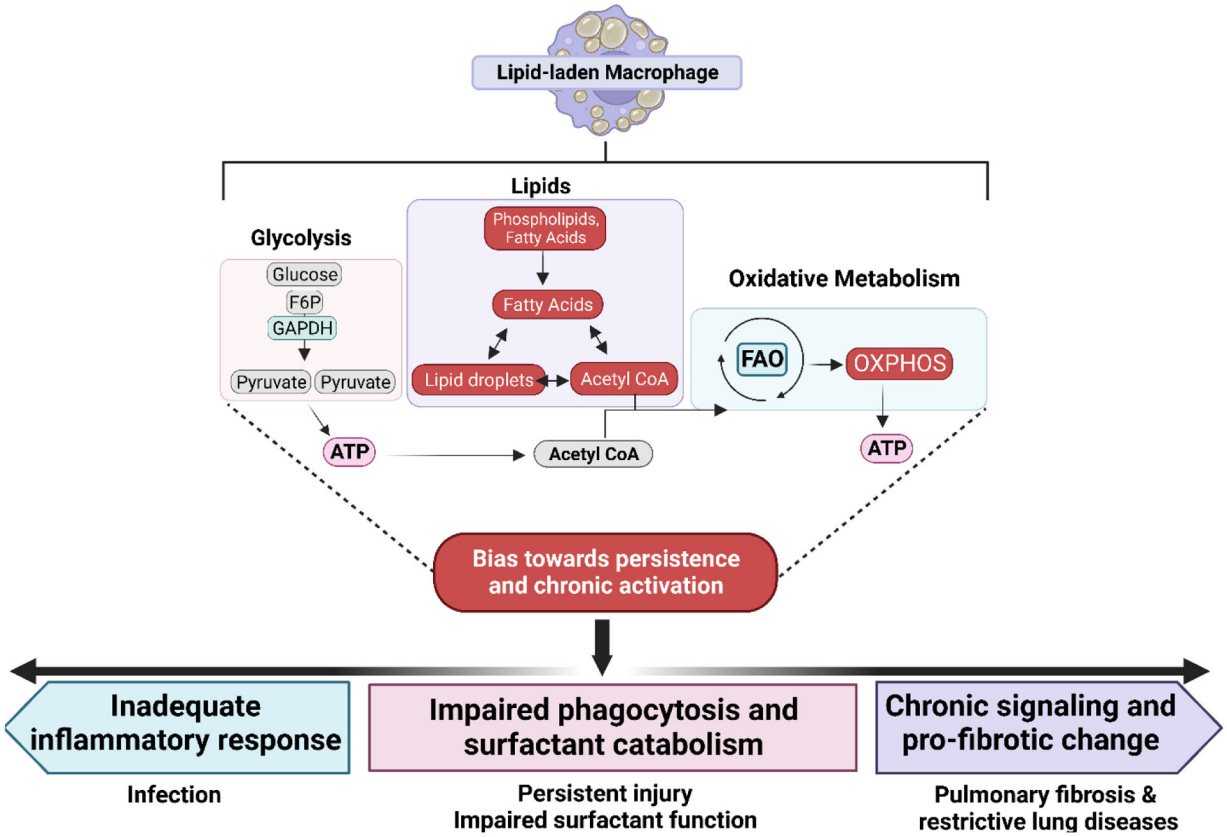

Figure 3.

Persistence of lipid laden macrophages in the lung may lead to dampened inflammation and fibrotic change. Due to excess lipid accumulation in macrophages of this phenotype, it is proposed that there is significant reliance on fatty acid oxidation (FAO) rather than glycolysis which is typical of an acute inflammatory response. Increased reliance on these processes is represented by red boxes. The downstream effects of these changes are proposed to bias towards chronic activation, which may ultimately lead to an inadequate inflammatory response upon stimulation, priming the lung for susceptibility to infection and persistent injury. Furthermore, this may impair the phagocytic capacity of the macrophage, leading to both ineffective inflammation and poor surfactant catabolism (pale blue box), further negatively impacting lung function. Persistence of this phenotype may also contribute to persistence of this traditionally “anti-inflammatory phenotype,” leading to pro-fibrotic signaling and collagen deposition in the lung (purple box), leading to fibrosis or other restrictive lung diseases.