A New Frontier in Immunometabolism

Cholesterol in Lung Health and Disease

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Abstract

The lung has a unique relationship to cholesterol that is shaped by its singular physiology. On the one hand, the lungs receive the full cardiac output and have a predominant dependence on plasma lipoprotein uptake for their cholesterol supply. On the other hand, surfactant lipids, including cholesterol, are continually susceptible to oxidation owing to direct environmental exposure and must be cleared or recycled because of the very narrow biophysical mandates placed upon surfactant lipid composition. Interestingly, increased lipid-laden macrophage "foam cells" have been noted in a wide range of human lung pathologies. This suggests that lipid dysregulation may be a unifying and perhaps contributory event in chronic lung disease pathogenesis. Recent studies have shown that perturbations in intracellular cholesterol trafficking critically modify the immune response of macrophages and other cells. This minireview discusses literature that has begun to demonstrate the importance of regulated cholesterol traffic through the lung to pulmonary immunity, inflammation, and fibrosis. This emerging recognition of coupling between immunity and lipid homeostasis in the lung presents potentially transformative concepts for understanding lung disease and may also offer novel and exciting avenues for therapeutic development.

Keywords: oxysterols; innate immunity; lipoproteins; liver X receptors

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In recent years, there has been a growing appreciation in the fields of vascular biology and endocrinology of the complex interactions that exist between cholesterol homeostasis and immunity. Cholesterol overload of different subcellular compartments in macrophages in varying contexts can induce endoplasmic reticulum stress–dependent cytokine production (1), augmented proinflammatory signaling by Toll-like receptors (TLRs) (2), inflammasome activation (3), or suppression of proinflammatory gene expression (4). Dynamic changes in cholesterol synthesis are also required for antiviral responses in macrophages and for proliferation of T cells (5). Although abnormal pulmonary accumulation of lipid-laden macrophage "foam cells" has been noted in several lung diseases, the mechanisms that regulate cholesterol levels in lung-resident cells, as well as the connection of these mechanisms to lung disease, remain poorly defined. In this minireview, a brief overview of recent advances in this area is presented. For a more detailed discussion of cholesterol trafficking in lung health and disease, the reader is directed to recent comprehensive reviews (6–9).

Reverse Cholesterol Transport: A Brief Primer for the Pulmonologist

Reverse cholesterol transport (RCT) is the homeostatic pathway by which tissue macrophages and other cells are protected from toxic cholesterol overload (Figure 1) (reviewed in [10]). This occurs through regulated cellular efflux and bodily elimination of cholesterol. At the cellular level, macrophage cholesterol content is controlled through the opposing actions of two families of transcription factors: (*1*) sterol response element–binding proteins (SREBPs), which induce target genes that promote cholesterol accumulation via uptake (low-density lipoprotein receptor) and synthesis (e.g., hydroxymethylglutaryl coenzyme A reductase); and (*2*) liver X receptors (i.e., LXR-α and LXR-β), which induce genes that reduce cholesterol uptake (e.g., inducible degrader of low-density lipoprotein receptor) and promote cholesterol efflux (e.g., the ATP-binding cassette transporters A1 and G1

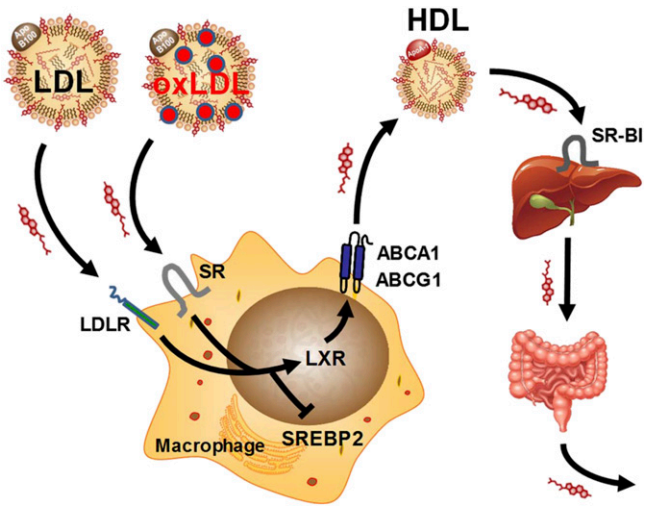

Figure 1. Reverse cholesterol transport pathway. Reverse cholesterol transport is the pathway by which tissue macrophages avoid toxic cholesterol overload. Cellular uptake of cholesterol from low-density lipoprotein (LDL) particles or oxidized LDL (oxLDL) particles via the LDL receptor (LDLR) or scavenger receptors (SRs), respectively, inhibits sterol response element–binding protein 2 (SREBP2) and activates liver X receptors (LXRs). The LXRs promote compensatory cholesterol efflux by upregulating ATP-binding cassette transporters A1 and G1 (ABCA1 and ABCG1, respectively). ABCA1 and ABCG1 together increase mobilization of cellular cholesterol to high-density lipoprotein (HDL) particles in the extracellular space. Plasma HDL delivers cholesterol to hepatocytes, where it is internalized via scavenger receptor BI (SR-BI). Cholesterol cleared from the plasma is then exported to the biliary system and from there to the intestinal lumen, where it can be excreted from the host. Apo B100 = apolipoprotein B 100; ApoA-1 = apolipoprotein A-I.

[ABCA1 and ABCG1, respectively]). Oxysterols (i.e., oxidized cholesterol, such as 25-hydroxycholesterol [25-HC] and 27-HC) serve as key integration signals between SREBPs and LXRs. Oxysterols accumulate during sterol overload and effect compensatory cholesterol reduction through activating LXRs (as direct agonists) and inhibiting SREBPs. Additional complex regulatory loops exist, including the intronic microRNA-33, which is cotranscribed with SREBP2 during low-cholesterol states and suppresses ABCA1 and ABCG1 (11). Ultimately, apolipoprotein A-I (apoA-I) and high-density lipoprotein (HDL) serve as plasma acceptors of cellular cholesterol effluxed via ABCA1 and ABCG1, respectively, and transport cholesterol to the liver for scavenger receptor BI (SR-BI)–mediated uptake and subsequent elimination via the bile into the feces.

RCT: Ancient Regulator of Immunity and Inflammation

Of interest, RCT regulates not only cellular cholesterol but also innate and adaptive immunity, as recently reviewed (5, 9, 10, 12). LPS, a glycolipid shed from the outer membrane of gram-negative bacteria, partitions into HDL in the plasma, where it is neutralized and then cleared via hepatic SR-BI, along with cholesterol undergoing RCT (10). This finding has suggested that plasma lipoproteins may be an ancient arm of host defense. Intriguingly, sequence homology between HDL-associated plasma proteins that bind microbial lipids (i.e., LPS-binding protein) and host lipids (e.g., phospholipid transfer protein), as well as host defense functions that have been identified for the latter, together support common ancestry between lipid homeostasis and innate immunity (10). Suggesting an intrinsic role for sterols in immunity, recent reports have also shown that several immune receptors are directly ligated and regulated by cholesterol (T-cell receptor-β) and oxysterols (C-X-C chemokine receptor type 2, G protein–coupled receptor 183) (5).

Complex cross-talk exists in macrophages between cholesterol mobilization and inflammatory functions of the cell (Figure 2). ApoA-I and HDL indirectly suppress proinflammatory signaling by TLRs and other receptors through reducing cholesterol in lipid raft microdomains of the plasma membrane (12). LXRs augment this effect through potentiating ABCA1-dependent raft cholesterol efflux (13) and inhibiting proinflammatory gene expression (14), whereas *Abca1*$^{-/-}$ and *Abcg1*$^{-/-}$ macrophages have augmented TLR responses as a result of raft cholesterol overload (2, 15). MicroRNA-33 also indirectly augments TLR responses by increasing raft cholesterol through targeting ABCA1 and ABCG1 (16). Conversely, inflammation has been shown to inhibit RCT at multiple steps in the pathway from peripheral macrophage to liver (17). Taken together, RCT and innate immunity appear to be coupled. RCT tonically suppresses inflammation but is itself also a target of inflammation, potentially feeding back during disease to de-repress innate immunity.

Good evidence has been amassed for RCT and its effect on immunity outside the lung. Until recently, however, it has remained unclear how cholesterol is regulated in the lung and whether RCT from the lung is linked to pulmonary immune responses and lung disease.

Cholesterol Homeostasis in the Lung?

Cholesterol is the major neutral lipid of pulmonary surfactant (7). Given that modest increases in cholesterol content compromise the surface tension–reducing properties of surfactant (18), it appears clear that cholesterol (and phospholipid) regulation must be a key and perhaps unique requirement of lung biology. Lipid tracer studies in rodents indicate that more than 80% of lung cholesterol is derived from the plasma (i.e., by uptake from lipoproteins) and the remainder, from synthesis in lung-resident cells (19). Plasma lipoprotein-derived fatty acids have also been shown to be incorporated within alveolar epithelial type 2 (AT2) cells into pulmonary surfactant phospholipid (20). Very low–density lipoprotein, LDL, and HDL all induce phosphatidylcholine secretion by AT2 cells (21, 22). Suggesting the possibility that dyslipidemia in humans may dysregulate surfactant lipid composition, researchers have shown that apoE-null mice (which have elevated

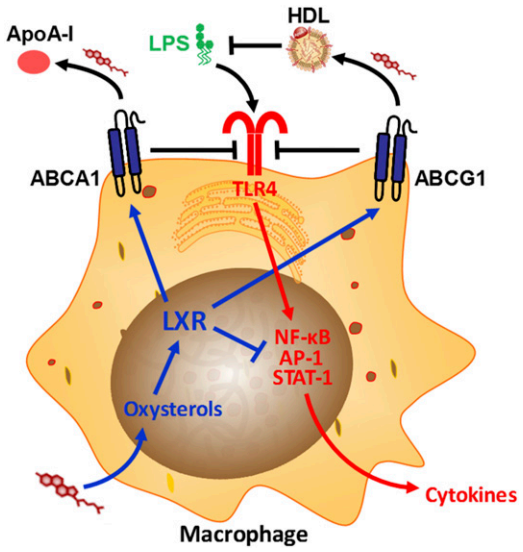

Figure 2. Interactions between cholesterol and proinflammatory Toll-like receptor 4 (TLR4) signaling in the macrophage. Oxysterols accumulate upon cholesterol internalization by the macrophage, activating liver X receptor (LXR). LXR up-regulates the cholesterol efflux proteins ATP-binding cassette A1 and G1 (ABCA1 and ABCG1, respectively) and inhibits proinflammatory cytokine induction by suppressing the activity of transcription factors (i.e., nuclear factor-κB [NF-κB], activator protein [AP]-1, and signal transducer and activator of transcription [STAT]-1) at gene promoters. ABCA1 and ABCG1 inhibit TLR4 activation in the plasma membrane through reducing lipid raft cholesterol (through promoting cholesterol efflux to the extracellular acceptors apolipoprotein A-I [ApoA-I] and high-density lipoprotein [HDL], respectively). HDL also directly suppresses lipopolysaccharide (LPS) signaling by binding and neutralizing LPS.

plasma very low-density lipoprotein and LDL) have abnormal surfactant (20). Interestingly, in addition to potential roles in promoting (retrograde) RCT from the lung, HDL serves as the major vehicle for anterograde delivery of the antioxidant vitamin E to AT2 cells (23) and also potentiates delivery of alpha-1 antitrypsin to the lung (24). Taken together, the lung, perhaps owing to its unique surfactant lipid requirements and highly oxidizing and protease-prone microenvironment, has complex requirements for coordinated delivery by plasma lipoproteins of lipid and protein cargo. Indeed, suggesting a fundamental dependence of lung biology upon lipid trafficking, the lungs of both HDL-deficient *Apoai*$^{-/-}$ mice and *Apoe*$^{-/-}$ mice have developmental and physiological abnormalities (25, 26). Specifically, the lungs of naive *Apoai*$^{-/-}$ mice display increased airway hyperresponsiveness, collagen deposition, and oxidative stress, whereas *Apoe*$^{-/-}$ mice have reduced developmental alveologenesis associated with accelerated loss of lung recoil with age.

Although authors of one report estimated cholesterol clearance from the air space to be very low (27), more recent genetic approaches have pointed to substantial steady-state flux of cholesterol through the lung. Thus, both *Abcg1*$^{-/-}$ and *Abca1*$^{-/-}$ mice have remarkable pulmonary overload with cholesterol and other lipids, along with abnormal accumulation of macrophage foam cells (28–31), suggesting that RCT prevents lipid overload in the lung. Cholesterol handling in the lung appears to be dependent on the maturity of alveolar macrophages insofar as granulocyte macrophage–colony stimulating–factor (GM-CSF)–null mice have ABCG1-deficient alveolar macrophages and pulmonary cholesterol overload (32). Suggesting that the same mechanisms are at play in humans, ABCG1-deficient alveolar macrophages and lung cholesterol overload are also found in patients with pulmonary alveolar proteinosis, a disease driven by neutralizing autoantibodies against GM-CSF (32).

LXR-deficient mice, which have defective RCT, also have increased foam cells in the lung (33). Interestingly, the enzyme that synthesizes the LXR agonistic oxysterol 25-HC (i.e., cholesterol-25-hydroxylase) is most highly expressed in the lungs of mice (34), whereas that which synthesizes the LXR agonists 27-HC and cholestenoic acid (i.e., cytochrome P450 family 27 subfamily A member 1) is highly expressed in the lung in humans (35). This may suggest that locally synthesized oxysterols play an important role in driving LXR-dependent RCT in the lung. The hydrophilic oxysterol cholestenoic acid is thought to play a particularly important role in mediating diffusional sterol export from the human lung; in humans, plasma levels of cholestenoic acid appear to derive largely from synthesis in alveolar macrophages (35). Given this, the finding that plasma cholestenoic acid declines in emphysema, sarcoidosis, and tuberculosis (35) may suggest a unifying mechanism by which lung RCT, as well as local LXR-dependent inflammation suppression, becomes compromised in chronic lung disease.

Increased lipid-laden foam cells have been documented in the lungs of smokers as well as in a wide range of chronic lung diseases (36, 37). Foam cells also increase in the lungs of mice after several experimental exposures, including cigarette smoke, silica, bleomycin, and radiation (38, 39). These findings suggest that pulmonary lipid dysregulation may be a common event in chronic lung disease. Deficient expression of ABC lipid efflux transporters has been noted in lavage cells from patients with sarcoidosis and in alveolar macrophages of mice after multiwall carbon nanotube instillation (40). In both cases, elevated cellular expression of microRNA-33 was noted, suggesting a potential unifying mechanism. Ozone has been shown to suppress LXR in respiratory epithelial cells by causing lipid adduction, suggesting a different mechanism through which environmental oxidants may impair lung RCT (41). Reduced apoA-I is found in bronchoalveolar lavage fluid of patients with idiopathic pulmonary fibrosis (42), pointing to deficient cholesterol acceptor capacity. Taken together, it is intriguing to speculate that defective RCT through a variety of mechanisms may possibly contribute causally in a final common pathway of lung disease pathogenesis and that, if so, its correction might be therapeutic. Outside the lung, plasma HDL has been shown to have a deficient cholesterol-mobilizing function in several systemic diseases, including obesity and diabetes mellitus (43, 44). This raises the

broader possibility that systemic metabolic disorders may also potentially impact lung-resident cells and lung disease phenotypes by compromising RCT.

Connections between Lipid Homeostasis and Immunity in the Lung

Recent work done at my laboratory and others has begun to identify intriguing connections in the lung between lipid homeostasis and immunity. Important roles have been shown for several RCT regulatory proteins, including ABCG1, SR-BI, LXRs, apoA-I, and apoE in pulmonary inflammation and host defense. In most cases to date, it has remained unclear to what extent abnormal cholesterol trafficking itself contributes causally to the immune phenotypes of mice in which these genes have been targeted. Nonetheless, reports that a high-cholesterol diet impairs pulmonary host defense against gram-negative bacteria and *Mycobacterium tuberculosis* (45, 46), reduces neutrophil trafficking to the inflamed lung (45), and augments airway eosinophilia in the ovalbumin model (47) do suggest that primary perturbations of cholesterol balance may be sufficient to alter pulmonary immunity.

Along with striking pulmonary lipid overload, *Abcg1*$^{-/-}$ mice display increased recruitment of multiple leukocyte subtypes to the lungs in the steady state, along with abnormal induction of air space cytokines and dysregulated maturation of pulmonary dendritic cells (48). Some of this likely arises from increased proinflammatory cytokine induction by *Abcg1*$^{-/-}$ alveolar macrophages, observed both in the steady state and after LPS stimulation (28, 29). Perhaps owing to accumulation of oxysterols and oxidized phospholipids, the lungs and pleural space of *Abcg1*$^{-/-}$ mice also exhibit local expansion of innate-like B1-b cells that overproduce natural antibodies targeting oxidation-specific lipid epitopes (49). This finding suggests that lipid handling in the lungs, which is normally managed by ABCG1, may play a direct and specific role in programming of the innate immune system. Indeed, *Abcg1*$^{-/-}$ mice display an enhanced host defense response during lung infection with *Klebsiella pneumoniae* (28), a more robust Th17/neutrophilic response to pulmonary allergen challenge (48), and an exacerbated pulmonary fibrotic response to bleomycin challenge (39).

Additional highly complex interactions that exist between lipid trafficking and pulmonary host defense are revealed by mice deficient in SR-BI, an alternate receptor for HDL perhaps best known for its role in RCT through mediating HDL uptake by the liver (Figure 1). Similarly to *Abcg1*$^{-/-}$ mice, *Scarb1*$^{-/-}$ mice (null for the gene encoding SR-BI [*SCARB1*]) exhibit increased neutrophil recruitment to the lungs after LPS exposure and bacterial infection (50). Much of this is likely driven by increased cytokine production by SR-BI–null alveolar macrophages and deficient SR-BI–dependent clearance of LPS from the air space. SR-BI deficiency in the adrenal glands also de-represses neutrophil trafficking to the infected air space by impairing adrenal production of antiinflammatory stress glucocorticoids, a process normally dependent upon SR-BI–dependent supply of HDL cholesterol to the adrenal cortex (51). Unlike *Abcg1*$^{-/-}$ mice, *Scarb1*$^{-/-}$ mice demonstrate dramatically increased bacterial overgrowth in the lungs and blood during pneumonia. This likely arises from impaired phagocytic killing, a defect that is independent of the dysregulated HDL cholesterol of the mouse (50). Taken together, SR-BI deletion reveals complex roles for coordinated trafficking of host and microbial lipids, both inside and outside the lung, in the integrated neutrophilic response to bacterial pneumonia. Because functional polymorphisms in human *SCARB1* have been identified that phenocopy many features of the *Scarb1*$^{-/-}$ mouse (52), it seems plausible that similar roles for SR-BI may be at work in the human lung.

The LXRs are expressed by alveolar macrophages and alveolar epithelial cells and also play context-dependent roles in the pulmonary innate immune response, as recently described (53). Specifically, treatment of mice with a synthetic LXR agonist reduces neutrophil accumulation in the air space induced by LPS inhalation and gram-negative bacterial infection. This appears to stem from suppression of air space tumor necrosis factor-α and inhibition of neutrophil chemotaxis and leads to overgrowth of bacteria in the lung and reduced survival. In contrast to this immunosuppressive effect of forced LXR activation, synthetic LXR agonist treatment has been shown to enhance host defense against *M. tuberculosis* (54) and the nonpulmonary pathogen *Salmonella typhimurium* (55). This stems from prosurvival and metabolic effects in infected macrophages (55, 56). In yet other settings, LXR-α has recently been shown to promote the fibrotic behavior of idiopathic pulmonary fibrosis fibroblasts and to increase lung fibrosis in the murine bleomycin model (57).

ApoA-I–null mice, which are HDL deficient, have exacerbated alveolar neutrophilia after both adaptive (i.e., ovalbumin sensitization and challenge [58]) and innate (i.e., LPS inhalation [59]) immune challenges. The former effect appears to arise from de-repression of air space GM-CSF and the latter through effects on neutrophil migration to the lung. A wide range of additional interesting pulmonary phenotypes have been noted in dyslipidemic apoE-null mice, including exacerbated neutrophilic lung injury (60), increased airway hyperresponsiveness and goblet cell hyperplasia after house dust mite exposure (61), emphysema while on a high-fat diet (62), and sarcoidosis-like lesions (63) and severe pulmonary hypertension (64) on a cholate-containing high-fat diet. The likelihood that apoE also differentially modifies lung disease in humans is suggested by the finding that mice engineered to express human *APOE2*, *APOE3*, or *APOE4* (the three major *APOE* alleles in humans) have differential disease severity in the house dust mite model of asthma (65). Furthermore, *APOE4*$^{+}$ human subjects, compared with those who are *APOE4*$^{-}$, have augmented *in vivo* innate immune responses that may derive from increased lipid raft cholesterol in circulating monocytes (66).

Although in many cases the precise mechanisms by which cholesterol trafficking regulates the pulmonary immune response remain unclear, authors of several clinical reports have found interesting relationships between serum cholesterol and lung disease in human populations. Thus, serum levels of HDL cholesterol and apoA-I are positively associated with FEV_1 among patients with atopic asthma, whereas serum LDL cholesterol is negatively correlated (67). Similar relationships between serum lipoproteins and FEV_1 were also noted in a U.S. national survey (68). By contrast, authors of a recent report found that higher HDL levels were associated with lower FEV_1/FVC ratio and more extensive radiographic emphysema among patients

with chronic obstructive pulmonary disease (69). Complex relationships of serum lipoproteins to both asthma and atopy have also recently been noted (70, 71). The potential for lung disease to impact serum cholesterol is also suggested by a recent report that a reduction in serum cholesterol may be a marker of disease progression in patients with nontuberculous mycobacterial lung infection (72).

Closing Remarks

Fundamental roles have been identified for cholesterol trafficking in regulation of the innate and adaptive immune response. Studies of gene-targeted mice have also revealed that there is substantial flux of cholesterol through the lung in the steady state and that this flux is linked in complex ways, likely direct and indirect, to immune homeostasis in the lung. Owing to the unique biophysical properties and environmental susceptibility of surfactant lipid, the alveolar space is almost certainly singular among body compartments in its requirements for and mechanisms of lipid homeostasis. To date, results of studies of cholesterol-reducing statins in several lung diseases, including acute lung injury, chronic obstructive pulmonary disease, and asthma, have unfortunately proven largely negative (73). However, future efforts in rodent models and ideally in patients need to more precisely profile the pulmonary lipidome, perhaps on a cell-type basis, during health and disease, as well as to better define how systemic metabolic perturbations such as obesity, insulin resistance, and dyslipidemia translate to metabolic changes in the lung. Targeted strategies such as this will almost certainly identify more specific opportunities for lipid metabolic therapy in lung disease. The continued emergence of new lipid-targeted strategies for lung disease therapy, such as apolipoprotein mimetic peptides (74), indeed suggests that we are in the very early days of realizing the full therapeutic potential of lipid reprogramming in lung disease. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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