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Roles of the Mevalonate Pathway and Cholesterol Trafficking in Pulmonary Host Defense

Kristin A. Gabor and Michael B. Fessler

Immunity, Inflammation and Disease Laboratory, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709, USA

Abstract

It has become increasingly recognized that cholesterol and lipoproteins play significant roles in both lung physiology and the innate immune response. It is now known that the innate immune response and the cholesterol biosynthesis/trafficking network regulate one another, with important implications for pathogen invasion and host defense. The activation of pathogen recognition receptors and downstream cellular host defense functions are critically sensitive to cellular cholesterol. Conversely, microorganisms can co-opt the sterol/lipoprotein network in order to facilitate their own replication. Given that over 50% of adults in the U.S. have cholesterol abnormalities and pneumonia remains a leading cause of death, the potential impact of cholesterol on pulmonary host defense is of tremendous public health significance. This review addresses the emerging link between the innate immune response and sterol homeostasis, with a focus upon implications for respiratory infection. Clinical translations, including in the area of potential therapeutic development for infectious lung disease, are also discussed.

Keywords

Cholesterol; Lipoprotein; Lung; Innate Immunity; Pneumonia; Statins

Lipid Science, Pulmonary Biology, and Host Defense: separate disciplines no more

Although the lung has not customarily been viewed as a cholesterol-sensitive organ, emerging literature has begun to reveal the pivotal importance of sterol trafficking to lung health and disease. In parallel, reports originating from the cardiovascular sciences have recently revealed that cellular sterols regulate the innate immune response. These two threads of literature have now converged upon the field of respiratory infection, together suggesting that cholesterol and its dysregulation (i.e., dyslipidemia) may be under-recognized determinants of pulmonary host defense in humans. In this review, after opening

Correspondence to: Michael B. Fessler, MD Immunity, Inflammation, and Disease Laboratory, National Institute of Environmental Health Sciences, National Institutes of Health, 111 T.W. Alexander Drive, P.O. Box 12233, Maildrop D2-01, Research Triangle Park, NC 27709, Ph: (919) 541-3701, Fax: (919) 541-4133.

Conflict of Interest

The authors state no conflict of interest.

with a brief overview of cholesterol trafficking, we discuss recent findings on how cholesterol trafficking and the proteins that regulate it critically impact lung physiology, phagocyte host defense functions, and host defense in the lung. We then examine the pathogen end of the equation, discussing new findings on cholesterol regulation of microbial pathogenesis/life cycle. Last, we close with a review of emerging data, basic and clinical, on how 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR) inhibitors (statins) impact pulmonary host defense.

Cholesterol Synthesis and Trafficking

For a detailed discussion of cholesterol homeostasis, the reader is directed to a recent comprehensive review [1]. Here, we provide a brief overview of cholesterol biosynthesis and trafficking, intracellular and extracellular (Fig. 1), as a starting point for discussing how sterol homeostasis impacts the lung and host defense. Cholesterol is a vital component of cell membranes and is required for embryonic development, as well as synthesis of steroid hormones and bile acids. Not surprisingly, disruption or dysfunction in cholesterol synthesis, storage, transport or removal leads to important disturbances in cell physiology.

Nucleated cells obtain cholesterol either via biosynthesis from acetyl-CoA (the mevalonate pathway) or by uptake via either the low density lipoprotein receptor (LDLR) or scavenger receptors (e.g., SR-A, CD36). Ultimately, the level of cellular cholesterol is regulated through the antagonistic actions of two families of transcription factors, the Liver X Receptors (LXRs) and the Sterol Response Element Binding Proteins (SREBPs) (Fig. 1). After transport through the endosomal system mediated in part via the Niemann Pick C (NPC) proteins, internalized cholesterol is enzymatically oxidized on side chain carbons, yielding oxysterols (e.g., 25-OH-cholesterol [25HC]). Excess oxysterols (denoting cellular sterol overload) are detected by LXRs. Upon activation, LXRs drive the expression of genes that promote cholesterol efflux (e.g., the sterol efflux transporters ATP Binding Cassette [ABC]A1 and ABCG1) and degradation of LDLR. Conversely, SREBPs are tethered in the endoplasmic reticulum during sterol excess, but are released and activated for transport to the nucleus during cellular sterol deficit, at which time they promote sterol accumulation by driving expression of LDLR and many of the enzymes of the mevalonate pathway, including HMGCR, the rate-limiting enzyme. In addition to cholesterol, the mevalonate pathway yields several bioactive products, including isoprenoids, heme A, dolichol, and ubiquinone.

At the organismal level, cholesterol and other lipids are transported in the plasma as cargo on lipoprotein particles. The liver, in response both to dietary cholesterol and hepatic cholesterol synthesis, produces and releases triglyceride-rich very low density lipoprotein (VLDL). VLDL is either cleared via apolipoprotein E (apoE)-mediated receptor binding, or processed, ultimately into LDL, by lipases and lipid-transfer proteins in the plasma. LDL, via apoB-mediated binding to LDLR, serves as the major vehicle for cholesterol delivery to peripheral tissues. The liver and intestines also produce high density lipoprotein (HDL), which, in large part due to its protein constituent apoA-I, promotes ABCA1/ABCG1-dependent efflux of cholesterol from peripheral cells. Following efflux, HDL-cholesterol is ultimately cleared by the liver via scavenger receptor B-I (SR-BI), and converted to bile

acids for transporter-mediated release into the biliary tract for fecal excretion. This completes the pathway of 'reverse cholesterol transport' from peripheral cells to feces.

Over the years, several lines of gene-targeted mice have been generated by the cardiovascular sciences in an effort to define the roles of cholesterol traffic regulators in cholesterol homeostasis and atherosclerosis. Thus, HDL-deficient *Apoa1*^{-/-} mice have revealed the importance of HDL to vascular biology; *ApoE*^{-/-} mice, which are hypercholesterolemic due to impaired lipoprotein clearance, are the most common murine model for atherosclerosis; and *Abcg1*^{-/-} mice have revealed the critical role of cellular cholesterol efflux in prevention of foam cell formation [1]. These studies have also shown that lipoproteins and cholesterol exert potent effects upon inflammation and immunity. Recently, these research tools and insights have been applied to the lung, and have begun to yield novel and exciting insights into pulmonary biology.

Importance of Cholesterol Trafficking to Normal Lung Physiology

It has been known for some time that the lung is responsive to circulating lipoproteins and their cargo. *In vivo*, LDL is taken up by the lung [2], and both LDL and HDL stimulate the hallmark function of alveolar epithelial type II cells, surfactant production [3]. Moreover, vitamin E is carried by HDL, and, through interactions with SR-BI, HDL in fact serves as the primary source of this antioxidant for alveolar type II cells [4]. Of interest, the canonical lung-protective elastase inhibitor alpha1-antitrypsin (AAT) is also carried on HDL particles. In a recent notable report, it was shown that intravenous administration of either AAT or HDL singly was moderately protective against elastase-induced emphysema in mice, but that injection with HDL-AAT complex conferred remarkable protection against emphysema and inflammation [5]. Given that HDL composition is modified during various common disease states (e.g., obesity, diabetes) [6], studies such as these raise the interesting possibility that systemic metabolic disorders may conceivably impact lung function via plasma HDL-dependent, SR-BI-mediated communications with lung-resident cells. Suggesting that plasma LDL cholesterol may also impact lung architecture and inflammatory status, a high-cholesterol diet in rabbits was shown to increase alveolar leukocyte and cytokine levels and alveolar septal thickness [7].

While one study has estimated the rate of cholesterol clearance from the airspace to be very low [8], the mechanisms by which cholesterol homeostasis is maintained in the lung remain largely obscure. The lung in fact faces several unique challenges when it comes to cholesterol. Cholesterol accounts for ~5–10% of surfactant mass, and it has been shown that dysregulation of surfactant cholesterol levels critically embarrasses the vital surface tension-reducing functions of surfactant [9]. Moreover, direct exposure of surfactant lipids to environmental oxidants (e.g., ozone, cigarette smoke) induces the formation of cytotoxic oxysterols and oxidized phospholipids. Cholesterol is susceptible to ozonolysis, yielding oxysterols, including 5 β ,6 β -epoxycholesterol (β -epoxide), that induce cell death in alveolar epithelial type I cells [10]. While the anatomic partitioning of LDL within the lung has not been clearly defined, oxidation of LDL in the lung (i.e., generation of oxLDL) has also been implicated in the pathogenesis of pulmonary edema [11].

Perhaps the most definitive advances confirming the importance of cholesterol trafficking to lung development and health have come from the lung phenotypes of gene-targeted mice. All of the cholesterol regulatory proteins depicted in Fig. 1 have been shown to be expressed in the lung, including apoE, apoA-I, ABCA1, ABCG1, NPC1, and NPC2. Remarkably, *ApoE*^{-/-} mice have reduced developmental alveologenesis and abnormal pulmonary function with increased airway resistance and static compliance [12]. *ApoA-I*^{-/-} null mice have increased airway resistance, inflammatory cell recruitment, and airway collagen deposition in the steady state [13]; suggesting translation of this finding to humans, HDL-cholesterol deficiency is also associated with reduced lung function in human populations [14]. Interestingly, mice null for NPC1, NPC2, ABCA1, and ABCG1 – all proteins known to play central roles in cellular cholesterol efflux – have revealed the importance of constitutive cholesterol mobilization to lung homeostasis. Thus, *Npc1*^{-/-} and *Npc2*^{-/-} mice display increased lung cholesterol, alveolar fluid lipidosis, thickened alveolar septae, and alveolar macrophage foam cells [15]. Similarly, both *Abca1*^{-/-} and *Abcg1*^{-/-} mice display marked pulmonary lipid overload, including in alveolar macrophages and alveolar epithelial cells; the latter strain also displays increased steady state recruitment of a wide variety of leukocyte types to the lung, suggesting that cholesterol may also tune inflammatory/immune status in the lung [16].

Effect of Cholesterol and its Trafficking on the Macrophage Innate Immune Response

Any discussion of regulation of pulmonary host defense by cholesterol first requires a review of the effects of cholesterol trafficking on host defense functions of phagocytes, in particular macrophages and neutrophils (Fig. 2). Recent studies have shown that activation of the Toll like Receptors (TLRs) in macrophages and other cells is critically sensitive to cellular cholesterol, likely due to the enhancing effects of cholesterol on localization of TLRs to lipid raft membrane microdomains [1]. Reduction of raft cholesterol with chemical tools attenuates LPS activation of TLR4 [17]. HDL also attenuates CD11b activation in human monocytes via inducing cholesterol efflux [18], and broadly suppresses pro-inflammatory gene expression via induction of the transcriptional regulator ATF3 [19]. Conversely, cholesterol loading of the macrophage plasma membrane itself induces TLR4-dependent responses, and cholesterol loading of late endosomes promotes TLR3-dependent signaling [20]. Macrophages with genetically induced cholesterol overload also display accentuated TLR responses. *Abca1*^{-/-} macrophages contain higher amounts of free cholesterol, TLR4, and TLR9 in lipid rafts, and have an enhanced proinflammatory response to LPS as well as to TLR2, TLR7 and TLR9 agonists [1, 21]. A similar albeit more pronounced enhancement of raft cholesterol and TLR signaling is observed in *Abcg1*^{-/-} macrophages [22]. Cholesterol overload in other compartments of the cell can elicit TLR-independent inflammation. For example, internalized cholesterol crystals induce NLRP3-dependent secretion of mature IL-1 β from human monocytes and macrophages [23], whereas free cholesterol overload of the endoplasmic reticulum induces ER stress-dependent cytokine production [24].

In addition to membrane effects, trafficking of cholesterol metabolites through the cell interior also impacts pro-inflammatory cell functions. In particular, LXRs, oxysterol-activated nuclear receptors that promote cholesterol efflux from sterol-overloaded cells (Fig. 1), regulate reciprocal control over cholesterol traffic and inflammation. LXRs repress pro-inflammatory gene expression in part through effects on NF- κ B [25] and, conversely, are themselves antagonized (in their target gene induction and promotion of cholesterol efflux) by activation of TLR3 or TLR4 during viral or bacterial infection [26]. LXR-null macrophages also show increased susceptibility to pathogen-induced apoptosis [25], and mice lacking LXRs are highly susceptible to infection with *Listeria monocytogenes* [27]. In human cells, activation of LXRs may augment TLR-driven cytokine and chemokine secretion [28], in part through directly inducing expression of TLR4 [29].

Oxysterols have been reported to modify inflammatory cellular functions through LXR-independent mechanisms as well. Of relevance to the lung, 25HC in particular has been reported to be increased in the sputum of chronic obstructive pulmonary disease patients [30], and also to be robustly induced by LPS in macrophages and dendritic cells [31]. In recent studies, 25HC has been assigned a startling number of roles in inflammation. In human bronchial epithelial cells, 25HC enhances cytokine induction by the TLR3 ligand polyinosinic:polycytidylic acid [poly(I:C)] [32]. 25HC is also reported to itself induce pro-inflammatory gene expression [33], to amplify inflammation by promoting the recruitment of AP-1 to the promoters of a subset of TLR-responsive genes [34], to reduce IL-1 β transcription and broadly inhibit IL-1-activation inflammasomes [35], to suppress mucosal (including airway) IgA by effects on B cells [31], to promote phagocytosis in macrophages [36], and to attract neutrophils via direct activity on the chemokine receptor CXCR2 [37].

Cholesterol Impacts Neutrophil Function

Neutrophils, key first-responder immune cells that traffic to the infected lung in response to chemokines produced by alveolar macrophages, have also been shown to be regulated directly and indirectly by cholesterol trafficking. Neutrophils from diet-induced hypercholesterolemic mice display blunted chemotaxis [38]. This may arise from actions of oxidized LDL (oxLDL) upon neutrophil chemokine receptors, as oxLDL itself acutely induces neutrophil chemotaxis *ex vivo*, whereas pretreatment of mice with oxLDL *in vivo* attenuates neutrophil migration to the lung in response to CXCR2-active chemokines [39]. Interestingly, HDL and apoA-I reportedly attenuate CD11b activation, adhesion, and migration of neutrophils, likely via reduction of raft cholesterol [40]. Synthetic LXR agonists have also been shown to attenuate neutrophil chemotaxis *ex vivo* as well as neutrophil migration to the inflamed lung [41], suggesting that oxysterols (endogenous LXR agonists) may impact neutrophilic lung disease via direct effects on circulating neutrophils. It was also recently shown that LXRs regulate steady state numbers of circulating neutrophils by promoting clearance of apoptotic neutrophils (efferocytosis) in the spleen via direct induction in phagocytes of the LXR target gene and efferocytosis receptor, Mertk [42].

Interestingly, emerging studies continue to find additional means by which effects of cholesterol on neutrophils can impact the lung. For example, two recent studies reported that

cholesterol crystal accumulation in neutrophils, and secondarily, in endothelial cells, may underlie the pathogenesis of transfusion-related acute lung injury [43].

Cholesterol Trafficking in Pulmonary Innate Immunity

Given that perturbations in cholesterol impact both lung homeostasis and phagocyte functions, it should come as little surprise that an emerging literature is now identifying mechanisms by which cholesterol trafficking impacts pulmonary host defense. Our group recently reported that mice fed a high-cholesterol diet (HCD) displayed attenuated influx of neutrophils into the airspace in response to LPS or *K. pneumoniae*, along with compromised bacterial clearance from the lung [39]. This reduced recruitment of neutrophils was due to impaired neutrophil chemotaxis and deficient induction of NF- κ B-dependent cytokines in the airspace. Interestingly, this phenotype appeared to be somewhat lung-specific, as, paradoxically, bacteria were cleared more effectively from the bloodstream and peritoneal cavity during dyslipidemia. In a similar vein, another group found that hypercholesterolemic *ApoE*^{-/-} mice were more susceptible to *M. tuberculosis* [44], and that this was further exacerbated by HCD, as evidenced by early mortality and increased lung inflammation and bacterial burden.

Suggesting a broad-spanning impact of apoE on innate inflammatory responses in the lung, we recently also reported that *ApoE*^{-/-} mice have enhanced neutrophil and monocyte/macrophage recruitment to the airspace in response to inhaled LPS, as well as enhanced airway neutrophilia in response to direct airway inoculation with CXCL1, whereas treatment of mice with an apoE mimetic peptide reduced leukocyte recruitment to the airway, likely through effects upon chemotaxis [45]. *ApoE*^{-/-} mice also have an impaired host defense against *K. pneumoniae* infection, with higher mortality and greater outgrowth of bacteria [46], as well as increased susceptibility to acute lung injury induced by mechanical or oxidant stresses [47], and increased susceptibility to endotoxemia [48]. Demonstrating potential relevance of variations in apoE function to human disease, our group recently reported that human subjects with an *APOE4* genotype have augmented pro-inflammatory responses to TLR ligands *in vivo* and *ex vivo* [49]. Whether such subjects have increased susceptibility to lung injury/infection is an interesting question that remains to be answered.

Intriguing roles have also recently begun to emerge for the cholesterol transporters ABCG1 and ABCA1 in pulmonary host defense. Our laboratory reported that ABCG1 negatively regulates innate immunity and antibacterial host defense in the lung [50]. *Abcg1*^{-/-} mice displayed markedly enhanced cytokine induction and neutrophil recruitment in the lung following exposure to LPS or *K. pneumoniae*, a response that arose in part from enhanced TLR responsiveness of alveolar macrophages. This was associated with enhanced bacterial clearance and reduced extrapulmonary bacterial dissemination. We are not aware of studies of the impact of ABCA1 on pulmonary host defense, but recently myeloid cell-specific ABCA1-null mice were shown to be more resistant to *Listeria monocytogenes*, displaying less weight loss, pathogen burden, and hepatic damage [51].

SR-BI, a receptor for HDL (Fig. 1), was recently identified as a receptor for mycobacteria in a study in which it was found that SR-BI^{-/-} mice have a significant reduction in production

of TNF, IFN γ , and IL-10 following infection with *M. tuberculosis* [52]. In a recent report from our laboratory, SR-BI^{-/-} mice were also found to suffer dramatically increased mortality during bacterial pneumonia with *K. pneumoniae*, associated with higher bacterial burden in the lung and blood as well as higher serum cytokines and increased organ injury [53]. SR-BI-deficient mice had significantly increased neutrophil recruitment and cytokine production in the infected airspace that was associated with reduced alveolar clearance of LPS as well as increased cytokine production by macrophages. Despite enhanced alveolar neutrophilia, SR-BI^{-/-} mice displayed impaired phagocytic killing, impairing host defense. Collectively, these studies identify multiple roles for SR-BI in host defense.

Complex roles for LXR in pulmonary host defense were recently reported by our group and others. Treatment of mice with a synthetic LXR agonist was found to compromise pulmonary host defense against *K. pneumoniae*, likely due to reduced alveolar neutrophil recruitment, a phenotype that was also observed with LPS inhalation [41, 54]. Another report found that LXR agonist-treated mice showed increased pulmonary expression of genes encoding antioxidant enzymes, along with resistance to LPS-induced lung injury and reduced oxidant production [55]. Interestingly, a protective effect of LXR agonist treatment was found in a mouse model of *M. tuberculosis* infection, and was linked to an increase of Th1/Th17 function in the lungs, whereas LXR-null mice displayed markedly increased susceptibility [56]. Lastly, in a rat model of hemorrhagic shock, treatment with a synthetic LXR agonist improved cardiac function and reduced lung injury and inflammation [57]. Taken together, these studies raise the possibility that targeting LXR may hold promise in manipulation of the lung's innate immune response.

Cholesterol in Bacterial Pathogenesis

In addition to regulation of host mechanisms by cholesterol traffic, several studies have identified roles for cholesterol in the pathogen life cycle (Fig. 2). Critical steps of infection such as colonization, replication, and dissemination can depend on the availability of host lipid. Moreover, bacteria have developed a variety of strategies to divert host lipids – or cellular processes driven by lipids – to their benefit. In some cases, lipids not usually present in prokaryotes have been described in several bacteria, likely a result of scavenging. Although lipids and lipoproteins play a key role in host defense, it is important to also recognize that microorganisms can co-opt the host lipid network to facilitate their own needs, as described in recent reviews [58].

Lipid rafts, cholesterol-enriched microdomains of the host plasma membrane, have been shown in multiple studies to play key roles in pathogen invasion into host cells (Tables 1–2). The importance of lipid rafts to bacterial invasion is exemplified by a study reporting that chemical agents that disrupt lipid rafts by targeting raft cholesterol blocked cellular invasion by *E. coli* and *P. aeruginosa* [59]. Microscopic images of bladder epithelial cells revealed that components of rafts (cholesterol, GM1, caveolin-1) were highly enriched in the membrane enveloping bacteria. Lipid raft aggregation was also found to play a role in the infection of type II alveolar epithelial cells (AECs) by *Mycobacterium tuberculosis*. Similar to the case with *Pseudomonas* and *E. coli*, treatment of host cells with a lipid raft-disrupting agent produced a reduction in viable bacteria [60]. Preincubation with inhibitors of lipid

rafts were used in another study to challenge primary AECs together with *Pneumocystis carinii* cell wall constituent B-1-3-glucan (PCBG) [61]. Inhibition of lipid rafts resulted in significant attenuation of *P. carinii*-induced expression of TNF α and MIP2. AECs were shown to internalize fluorescently labeled PCBG by raft-mediated mechanisms and inhibition of rafts prevented internalization, together suggesting a role for AEC raft function in inducing inflammatory responses to PCBG. In still another study of host plasma membrane manipulation, Korhonen et al. examined the invasion and attachment of *Chlamydia pneumoniae* into nonphagocytic epithelial cells [62]. Treatment with cholesterol-sequestering reagents inhibited *C. pneumoniae* infection, suggesting that the attachment and invasion of this bacterium is dependent on the formation of cholesterol-rich lipid rafts. A recently developed model system using mouse embryonic fibroblasts (MEFs) deficient in 24 sterol reductase, the terminal enzyme in the cholesterol biosynthetic pathway, enabled a targeted approach to investigating the role of cholesterol synthesis in pathogen invasion [63]. In the absence of cholesterol, *Coxiella burnetii* entry was decreased, but infection by *Salmonella typhimurium* and *Chlamydia trachomatis* was unaffected, suggesting a role for lipids rafts in *C. burnetii* uptake.

In addition to lipid rafts, other cholesterol-dependent membrane microdomains have roles in microbial pathogenesis. Caveolae are morphologically distinct membrane microdomain structures that, similar to rafts, are enriched in cholesterol. Caveolae require caveolin-1 (cav-1) and cav-2 as scaffolding proteins for signaling molecules that associate with caveolae and that regulate caveolar functions such as endocytosis and cell signaling. Caveolae have been implicated in cellular invasion for multiple pathogens (Tables 1–2). A role for the caveolin proteins in the pathogenesis of *Pseudomonas aeruginosa* was reported in a study that demonstrated that Cav-1-null mice were resistant to infection in lung epithelial cells, while their wild-type counterparts succumbed to infection [64]. In addition, invasion of type I AECs was inhibited by cholesterol-disrupting agents, an effect which was restored with the addition of cholesterol to the cell membrane [65]. Ceramide molecules can also form distinct domains in the cell membrane which, like the other membrane domains, organize cellular receptors and signaling molecules [66]. Ceramide is required for internalization of *Neisseriae gonorrhoeae* and is also a critical trigger for death in *Staphylococcus aureus*-infected endothelial cells. Interestingly, *Pseudomonas aeruginosa* infection results in the release of ceramide and the formation of ceramide-enriched membrane domains. *P. aeruginosa* then localizes to these domains and infects the cells via ceramide-enriched membrane domains, inducing apoptosis and regulating the cytokine response in infected cells [66–67]. Genetic deficiency of acid sphingomyelinase prevented internalization of bacteria, induction of death in infected cells, and a controlled release of cytokines in the infected lung due to abrogated formation of ceramide-enriched membrane domains [66]. Failure to form ceramide-enriched membrane domains in *P. aeruginosa*-infected cells results in a hyperinflammatory response, massive release of IL-1 β , and septic death of mice [67].

Interestingly, *M. tuberculosis* resides in lipid-laden foam cell macrophages during its dormant stage of *in vivo* infection [68], and is now recognized to require host cholesterol at various stages of its infectious life cycle [69]. Mycobacteria in fact have a large regulon of cholesterol metabolic genes [70]. Metabolism of cholesterol via mycobacterial cholesterol

oxidase is required for intracellular replication and for anti-inflammatory actions of the bacterium that subvert phagocyte immunity [71]. Studies such as these suggest that hypercholesterolemia as well as cholesterol-targeting therapeutics may possibly exert indirect and clinically relevant effects on mycobacterial infectivity.

Cholesterol in Viral Pathogenesis

Cholesterol also plays an essential role in the life cycle of viruses, many of which manipulate host cholesterol metabolism to facilitate their own replication and life cycle (Table 2). A prevalent respiratory pathogen that targets people of all ages is respiratory syncytial virus (RSV). A recent report demonstrated that cholesterol-rich membrane microdomains are used by RSV for docking to primary normal human bronchial epithelial cells, that cholesterol in the cell membrane is essential for RSV to successfully infect bronchial epithelial cells, and that cholesterol depletion of cells inhibits RSV infection [88]. Lipid rafts were additionally found to be required by RSV for release of infectious virus particles, as treatment of human lung epithelial cells with raft-disrupting agents led to diminished RSV infectivity resulting from an inhibition in the release of infectious progeny virion particles [89]. Similarly, using a genetic approach, it has been shown that human NPC syndrome fibroblasts, which have abnormal rafts due to defective cholesterol trafficking, also have reduced release of infectious RSV virions, resulting in a significant reduction in RSV infectivity [89].

Budding of influenza A virus (IAV) from the plasma membrane is also dependent on the presence of lipid rafts. The two major viral glycoproteins, hemagglutinin (HA) and neuraminidase (NA), associate with lipid microdomains and purified IAV particles contain high levels of cholesterol [90]. Pretreatment of IAV virions with the cholesterol-extracting chemical methyl- β -cyclodextrin (M β CD) depleted envelope cholesterol from IAV and reduced infectivity in a dose-dependent manner, an effect which could be partially rescued by addition of exogenous cholesterol. Depletion of envelope cholesterol also reduced IAV fusion. Recently, elevated levels of cellular annexin A6 were shown to reduce the infectivity of progeny IAV particles by increasing late endosomal cholesterol and decreasing plasma membrane cholesterol, thereby impairing IAV replication and propagation [91]. Conversely, the effects of IAV infection on cholesterol homeostasis have recently also been shown. Of interest, IAV infection induces pulmonary expression of cytosolic sulfotransferase (SULT)2B1b, an enzyme that inactivates the LXR activity of oxysterols through sulfation. It thus appears possible that IAV may impact pulmonary immunity and lipid homeostasis through metabolic dysregulation of the LXR pathway [92].

Numerous studies now indicate that cholesterol plays an essential role in human immunodeficiency virus type 1 (HIV-1) infectivity through multiple mechanisms, including viral budding from lipid raft microdomains. Direct depletion of cholesterol from HIV-1 envelopes reduces virus infectivity through impacting viral entry into the cell [93]. Suggesting that HIV-1 has co-opted cholesterol trafficking to the benefit of its life cycle, HIV-1 Nef protein augments the cholesterol content of virus particles and thereby their infectivity by inhibiting cellular cholesterol efflux via downregulation of host ABCA1 [94].

Conversely, 25HC, an oxysterol LXR ligand that is induced by host cells after TLR3 activation [31], was recently shown to suppress HIV-1 replication. SREBP2 is activated by HIV-1 infection of CD4⁺ T cells, but is well known to be inhibited by 25HC and other oxysterols. Treatment of T cells with 25HC was shown to reduce induction of TFII-I, a key gene regulating HIV transcription. Using small interfering RNA, silencing of SREBP2 or TFII-I was shown to reduce HIV-1 production in CD4⁺ T cells, together demonstrating that HIV-1 infection in T cells is intrinsically linked to and dependent upon cholesterol homeostasis. In a similar vein, the synthetic LXR agonist T0-901317 restored cholesterol efflux from HIV-1 infected T lymphocytes and macrophages and potently suppressed HIV-1 replication in both cell types, through a mechanism that was shown to rely on the LXR target gene ABCA1[95]. Reduced viral cholesterol was connected to reduced fusion activity of the virions and, ultimately, to a defect in infectivity.

Although not a lung pathogen, several studies have shown that hepatitis C virus (HCV) subverts cholesterol trafficking for key processes throughout its life cycle. SR-BI is now recognized as a receptor for HCV and is under active study as a therapeutic target in this application [96]. In addition, persistent overexpression of DHCR24 was found in the livers of HCV-infected patients as well as chimeric mice with human hepatocytes, whereas silencing or inhibition of DHCR24 decreased HCV replication, suggesting the potential of this cholesterol synthetic enzyme as a novel druggable anti-HCV target [97]. Several components of sterol pathway including SREBP2 have also been found to be important for infection by Andes virus. Using a variety of approaches, Petersen et al. [98] showed that disruption of sterol regulatory function impaired internalization, but not virus binding. Andes entry was sensitive to changes in cellular cholesterol. Lastly, and of timely public health relevance, infections by the Ebola and Marburg filoviruses were connected to mutations that disrupt the NPC1 cholesterol transporter protein [99]. Human primary fibroblasts derived from NPC1 patients were resistant to Ebola and Marburg infection, while remaining susceptible to a plethora of unrelated viruses. Overexpression of human NPC1 reversed the resistance to infection, demonstrating a crucial role for a cholesterol transport protein in filovirus infection.

Many other viruses exploit cholesterol to facilitate their life cycle. Flaviviruses Dengue and West Nile Virus show sensitivity to perturbation of cholesterol biosynthesis. Pretreatment of virions with M β CD reduced dengue virus infectivity in a dose-dependent manner [100]. In a study that demonstrated how host cell cholesterol homeostasis can be physically manipulated by viruses, West Nile virus (WNV) was shown to upregulate cholesterol biosynthesis and redistribute cholesterol to viral replication membranes [101]. Manipulation of cholesterol levels abrogated virus replication. In a key discovery, the authors further demonstrated that the WNV-induced redistribution of cellular cholesterol downregulated the interferon-stimulated Jak-STAT antiviral response, which could only be partially rescued with exogenous cholesterol. This demonstrates that WNV not only co-opts cholesterol for its own replication membranes, but thereby also subverts the host defense response.

Cholesterol Trafficking in Antiviral Responses

Besides exploiting host cell machinery for replication, viruses must also circumvent the interferon (IFN)-dependent cellular immune response. Upon invasion, recognition of viral products by pattern recognition receptors triggers the induction of transcription factors involved in expression of IFN [117]. IFN then initiates a signaling cascade via the Jak-STAT pathway, culminating in the production of antiviral products known as IFN-stimulated genes (ISGs) [117a].

Subversion of the IFN response via cholesterol modulation and redistribution was discussed above in a study of WNV [101]. Another example of viral evasion of the IFN response was recently reported in a study in which a rhabdovirus was shown to downregulate Cav-1, inhibiting the ability of IFN receptor molecules to cluster in cholesterol-enriched caveolae domains and induce an effective downstream antiviral response [118]. Negative regulation of the mevalonate pathway upon viral infection or cytokine treatment with IFN γ or IFN β further demonstrates an intrinsic link between the antiviral response and the sterol metabolic network. Thus, using conditioned media from infected cells, it was recently shown that secreted IFNs signal via the receptor IFNAR1 to downregulate enzymes in the mevalonate pathway in a manner that is inhibitory to virus [119]. Pharmacologic and RNAi inhibition of the mevalonate pathway was protective against viral infection of cells in culture and in mice, confirming a fundamental link between host sterol metabolism and the IFN antiviral host response. Further insight on the impact of sterol trafficking on the antiviral response was recently provided by investigators who showed that the antiviral effector protein IFN-inducible transmembrane protein 3 (IFITM3) impairs cholesterol homeostasis and viral entry by disrupting interaction between vesicle-membrane-protein-associated protein A and oxysterol-binding protein, two regulators of intracellular cholesterol trafficking [120]. Finally, and of translational interest, 4F, a mimetic peptide for the cholesterol trafficking apolipoprotein A-I, has shown some early promise as a potential IAV therapeutic. 4F displays cytoprotective and anti-inflammatory activity in an IAV-infected alveolar epithelial cell line [121] and also reduced systemic and intravascular inflammation and lung viral titers in a murine model of IAV infection [122].

Recently, cholesterol-25-hydroxylase (Ch25h) was shown to be an ISG in virus-infected cells, and its oxysterol product, 25HC, was shown to have potent antiviral activity [123]. Treatment of cultured cells with 25HC inhibited infections with a broad array of viruses (e.g. VSV, HSV, HIV, Ebola, and others) via suppression of fusion between virus and cell, and potentially through additional effects. Notably, Ch25h-deficient mice were more susceptible to lytic infection by murine gammaherpesvirus and HIV-1 replication could be suppressed by administration of 25HC. Thus, taken together, broad-spanning effects of IFNs on sterol and oxysterol synthesis may be key events in the antiviral response.

A role for statins in host defense?

In the wake of mechanistic reports described above showing that cholesterol regulates both host and pathogen during infection have come applied studies seeking to define whether pharmacologic intervention upon sterols can be used to therapeutic benefit. The final section

that follows below illustrates emerging evidence that HMGCR inhibitors ('statins') in particular impact both host and pathogen during infection, and hold some promise as adjunctive therapeutics during infection.

Statins have been under intense study for well over a decade for their therapeutic potential in innate immunity. Through targeting of HMGCR, statins deplete cellular stores of multiple mevalonic acid-derived cellular metabolites, including cholesterol, isoprenoids (geranylgeranyl-pyrophosphate, farnesyl-pyrophosphate), dolichol, heme A, and ubiquinone. Most effects of statins on cell signaling and function are thought to arise from depletion of isoprenoids, lipids that play a key role in membrane-localization of specific proteins (e.g., Rho GTPases) through post-translational modification ('prenylation') [124]. On the other hand, statins have also been reported to inhibit phagocytosis and signaling via cholesterol depletion [125], and to inhibit LFA-1-dependent cell adhesion and co-stimulation through a mechanism independent of the mevalonic acid pathway altogether [126]. Multiple signaling proteins have been shown to be inhibited in statin-treated cells, including mitogen-activated protein kinases, Akt, Rho GTPases, and NF- κ B, and this is associated with reduced cell expression of cytokines and adhesion molecules [127]. A comprehensive review of statins in inflammation is beyond the scope of this review; we instead focus on major findings relating to the impact of statins on the innate immune response during encounter with pathogens.

Anti-inflammatory mechanisms of statins in the innate immune response

Several reports have now shown that treatment of lung epithelial cells, macrophages, and other cells with statins attenuates pro-inflammatory responses to LPS, including NF- κ B activation and cytokine induction [128]. Several mechanisms have been proposed, including regulation of TLR4 expression [129]. Of interest, some reports have on the other hand shown that statins enhance LPS responsiveness of cells, leading to increased production of IL-12p40 [130]. Statins have also recently been shown to promote caspase-1-dependent processing of IL-1 β [131] through increasing mitochondrial reactive oxygen species [132], and enhancement of NLRP3-dependent inflammasome activation by statins has been further linked to aggravated lung injury in a murine model [132].

Effects in cell culture notwithstanding, statins have clearly been shown to attenuate the innate immune response *in vivo*. Statins inhibit LPS-induced acute lung injury in murine models through reducing neutrophil trafficking [133] and vascular leak [134]. Protection from LPS-induced lung injury has been linked to upregulation of claudin-5 and integrin- β 4 on lung endothelial cells [135], and also to inhibition of LPS signaling by macrophages due to upregulation of the tetraspanin CD9 [136]. Suggesting relevance to humans, simvastatin is reported to attenuate lung inflammation induced by LPS inhalation in human volunteers [137], and plasma cytokines induced by intravenous LPS in human volunteers [138].

In addition to inhibition of the innate immune response, statins have been shown to have inflammation pro-resolving activity. For example, lovastatin triggers the biosynthesis of the anti-inflammatory and pro-resolving mediator 15-epilipoxin A4 by promoting transcellular communications between neutrophils and epithelial cells [139]. Statins also promote apoptosis of neutrophils in the human airway [137, 140] as well as anti-inflammatory clearance of apoptotic neutrophils by macrophages [141]. One randomized, double-blinded,

placebo-controlled trial found that simvastatin was associated with improvements in non-pulmonary organ dysfunction in acute lung injury patients [142]. This said, enthusiasm for statins as potential acute lung injury therapeutics was recently reduced by the reporting of a large multicenter ARDS Clinical Trials Network study which found that rosuvastatin did not improve mortality or ventilator-free days and was instead associated with fewer days free of renal and hepatic failure [143].

Impact of statins on antimicrobial cell functions

Given that the pro-inflammatory and host defense functions of phagocytes are virtually inseparable, this has led to questions about possible effects of statins on host defense against pathogens. A growing literature has now begun to reveal complex actions of statins on antimicrobial cell functions. Interestingly, several reports indicate that statins inhibit phagocytosis of bacteria [125, 144] and oxidative burst [144], while others suggest that, at least at high concentrations, statins may enhance phagocytosis [145]. Effects on phagocytosis have variably been found to arise from isoprenoid [144a] or cholesterol [125] depletion. Statins have also been shown to impair opsonophagocytic killing of bacteria [133, 144a]. On the other hand, one recent report indicates that statins may enhance phagocyte extracellular killing of *S. aureus* by promoting release of extracellular traps from neutrophils and monocytes/macrophages [144b]. This has even led to an ongoing trial to test whether statins might serve as adjuvants to improve neutrophil antibacterial infection in older infected patients [146].

Direct and indirect antimicrobial actions of statins

Several reports have now shown that statins themselves possess antibiotic activity, although in most cases the concentrations tested have had been far higher than are encountered *in vivo*. HMG CoA reductase is in fact expressed by prokaryotes, although the bacterial enzyme has $\sim 10^4$ -fold reduced binding of statins compared to the human enzyme [147]. This, as well as negative mevalonic acid rescue studies [148], together suggest that statins do not compromise bacterial growth through inhibition of HMG CoA reductase. Simvastatin has a minimum inhibitory concentration of 29.2 mg/L against *S. aureus*, whereas fluvastatin is significantly less potent [149]. The *in vivo* relevance of this is however far from clear as the typical peak plasma concentration of simvastatin in volunteers after a 40 mg oral dose is reported to be 0.0209 mg/L [150]. Moreover, while the MIC of simvastatin against *S. pneumoniae* and *H. catarrhalis* is 15 μ g/ml, single doses given to healthy volunteers do not improve the antibacterial activity of whole blood [148]. Of interest, HMG-CoA reductase-dependent production of ergosterol is also critical to fungal membrane integrity and statins have been shown to have efficacy against multiple fungal strains, although typically also at supraphysiologic concentrations [151].

In some cases, indirect inhibitory actions of statins on intracellular pathogen growth have been identified. Thus, simvastatin promotes macrophage killing of *L. monocytogenes* by reducing phagosomal membrane cholesterol, thereby compromising listeriolysin O (a cholesterol-binding cytolysin)-dependent escape of the pathogen into the cytosol [152]. In a related vein, statins reduce lung injury from pneumolysin-dependent cell lysis in a model of pneumococcal pneumonia [153]. Statins promote reduction of intracellular *M. tuberculosis*

burden by human peripheral mononuclear cells by enhancing phagosomal maturation and induction of autophagy [154]. Lovastatin inhibits intracellular replication of *C. burnettii* likely through reduction of cholesterol in the parasitophorous vacuole membrane, potentially at concentrations achievable in the serum of patients [155]. Similar results are found with *R. conorii* infection [156]. Statins also inhibit intracellular replication of *Salmonella* within macrophages, leading to reduced infectious burden *in vivo* in mice [157].

Several reports have now also shown that some statins inhibit the life cycle of viruses through actions on host cells. Thus, lovastatin treatment of mice diminishes replication of respiratory syncytial virus and decreases weight loss and illness measures even when given 24 hours after viral inoculation [158]. Fluvastatin inhibits the life cycle of cytomegalovirus in human endothelial cells [159]. Statins are also reported to disrupt replication of hepatitis C virus in cells, potentially through a mechanism involving depletion of geranylgeranyl-pyrophosphate [160]. Statins also impair particle assembly of rotavirus and dengue virus [161], and viral load of and cell entry by HIV-1, the latter through blocking geranylgeranylation-dependent activity of Rho [162]. Collectively, although the *in vivo* relevance of statin antimicrobial activity is in most cases uncertain, these exciting reports do at least support the idea that statins should be examined as potential adjuvant treatments during infection.

Impact of statins on sepsis and pneumonia: animal models

The success of host defense *in vivo* is a tenuous balance between mounting an immune response that is robust enough to clear invading pathogens, but not so exuberant as to critically compromise or even kill the host. A variety of statins have now been studied in murine *in vivo* models of sepsis and pneumonia. An early study by Ando and colleagues nearly 15 years ago showed that cerivastatin improves survival in a model of LPS-induced sepsis [163]. In the more complex and physiologically relevant sepsis model of cecal ligation and puncture, it has more recently been shown that simvastatin improves survival, at least in part through preserving cardiac function and hemodynamic status [164]. Protection is in fact seen with several statins, even when treatment is commenced 6 hours after sepsis induction, a time point at which hemodynamic compromise has already commenced [165]. Simvastatin also reduces mortality in an intravenous inoculation model of *S. aureus* bacteremia [166].

Studies of statins in murine models of pneumonia have yielded a range of results, collectively suggesting that the specific statin and infecting pathogen are important variables. Our group reported several years ago that lovastatin leads to impaired pulmonary clearance of the Gram-negative bacterium *K. pneumoniae*, and that this may derive from inhibitory effects on neutrophil trafficking and killing function [133]. On the other hand, it is reported that simvastatin improves mortality from *S. aureus* pneumonia whether [167] or not [166] antibiotic co-treatment is delivered, and that simvastatin leads to improved pathogen clearance in the lung despite reduced pulmonary recruitment of neutrophils [166]. Simvastatin is also reported to reduce lung injury and improve pathogen clearance in a murine model of pneumococcal pneumonia, although with no significant effect on survival [168]. In a model of pneumococcal pneumonia involving a mouse model of sickle cell disease, however, simvastatin treatment reduced mortality, lung injury, and bacterial burden

in lungs and blood [153]. Interestingly, this was attributed, at least in part, to reduced expression of the platelet activating factor receptor – a receptor utilized by *Pneumococcus* for cell invasion – as well as reduced pneumolysin O-induced cell injury [153]. Simvastatin and rosuvastatin are also reported to reduce bacterial dissemination and lung histopathologic changes in a *M. tuberculosis* lung infection model [154]. Finally, statins have been examined in murine models of influenza A pneumonia. Collectively, it has been shown that several statins have no significant effect on viral clearance, lung injury, weight loss, or mortality [169], although one group found reduced survival in simvastatin-treated mice [170].

Evolving data on statins in human infection: a complex and unfinished story

In 2001, Liappis and colleagues reported, in a single-center retrospective analysis of cases of Gram-negative rod or *S. aureus* bacteremia, that statins (i.e., patients who were admitted on, and continued taking statins) were independently associated with reduced mortality [171]. This was followed shortly afterward by corroborating reports, including a prospective observational cohort study by Almog and colleagues in 2004 that found that statins were associated with reduced progression to severe sepsis and ICU admission among patients admitted with acute bacterial infection [172]. Since then, a profusion of publications, most of them observational and several of them meta-analyses, have appeared that have addressed potential roles for statins in a wide range of infectious disease contexts. These have ranged from prophylaxis and treatment of (assorted) infections, to sepsis, community-acquired pneumonia (CAP), and ventilator-associated pneumonia (VAP). Despite many observational studies that have found reduced pneumonia incidence and improved pneumonia outcomes with statins, substantial doubt has lingered in the field due to the possibility of unmeasured confounding by a ‘healthy user effect’ – the idea that those patients who are prescribed statins are also likely to have increased rates of a variety of other health-promoting exposures and behaviors.

Complicating matters, statins have salutary effects beyond immunomodulation that need to be considered. Given that the risk of myocardial infarction and stroke is acutely increased following respiratory tract infection [173] and that statins have been linked to decreased risk of cardiovascular complications in inpatients with CAP [174], there may be a good rationale beyond host defense for using statins in infected patients. A comprehensive review of this literature is beyond the scope of the present chapter; instead, salient findings of the field on the impact of statins on human clinical infectious disease are discussed below.

A meta-analysis of observational studies published through 2007 on statins in a wide range of human infections (bacteremia, pneumonia, sepsis) found that statin use was associated with a reduction in both the incidence of infections as well as in complications/mortality arising during the treatment of infections [175]. Evidence, however, was found for both between-study heterogeneity and publication bias [175]. Similarly, a population-based cohort analysis of 141,487 patients admitted for a vascular event (acute coronary syndrome, stroke, revascularization) found that, after propensity matching for the likelihood to receive a statin, statin prescription was associated with a reduction in the incidence of subsequent sepsis [176]. By contrast, other studies have had dissenting findings. Thus, a meta-analysis of randomized placebo controlled trials of statins published through March, 2011 (11 trials,

totaling 30,947 participants), found no effect of statins on the risk of infections or infection-related deaths [177]. Moreover, a retrospective cohort study involving over 45,000 patients recently found that statin use was associated with an increased incidence of common infections (respiratory, skin, urinary tract, bacteremia)[178]. Two studies, one observational [179] and the other prospective, randomized, double-blinded and multicenter [180] also recently found that early prescription of a statin after presentation with an ischemic stroke is associated with a significant increase in the development of new infections.

In the context of sepsis, a variety of clinical outcome effects have been associated with statins. A meta-analysis of the literature through 2009 that involved a total of 20 studies of patients with sepsis and other acute infections found that statins were associated with a significant reduction in multiple measures of mortality [181]. A recent phase II, randomized, double-blinded, placebo-controlled trial of 100 statin-naïve patients presenting with sepsis found that treatment with atorvastatin 40 mg daily was associated with a significantly reduced rate of conversion to severe sepsis; however, no effect on length of stay, ICU admission or mortality was observed [182]. A 2013 meta-analysis of randomized controlled trials on statin therapy in septic patients (5 trials involving 650 patients) also found no significant effect of statins on mortality or hospital length of stay [183]. A recent phase II, multicenter, prospective, randomized, double-blind, placebo-controlled trial of patients with severe sepsis found no effect of atorvastatin treatment on plasma IL-6 concentration (primary endpoint), as well as no effect on length of stay, change in organ failure scores, or mortality [184]. However, of interest, in a predefined group of 77 prior statin users, those randomized to placebo (i.e., discontinued from their statin upon admission) had greater 28-day mortality than those treated with atorvastatin. While this might support continuation of statins upon hospital admission for infection, another prospective trial of patients hospitalized for infection and on pre-existing statin therapy found no difference in progression of sepsis nor hospital mortality whether or not patients were continued on statins in the hospital [185]. Taken together, compelling evidence to support statins in sepsis treatment is lacking, as is confident guidance on whether to continue statins during critical illness.

Two prospective, randomized, controlled trials have been published to date of statins in relation to VAP [186]. In the first, a two-center, open-label trial in 152 mechanically ventilated patients, pravastatin treatment was associated with a significantly increased probability of being free from VAP while in the ICU, and with reduced ICU mortality [186a]. By contrast, a randomized, placebo-controlled, multicenter trial of simvastatin in mechanically ventilated patients with suspected VAP was recently stopped early for futility with simvastatin being associated with a numerically compelling albeit not quite statistically significant increase in day-28 mortality, especially among formerly statin-naïve patients (mortality, 21.5% with statin vs. 13.8% with placebo; $P=0.054$) [186b].

Finally, no less controversy surrounds statins in the context of CAP, a clinical setting in which concerns about a ‘healthy user effect’ have most often been voiced. A recent meta-analysis of studies of statin treatment during CAP (13 studies involving 254,950 patients) found that statins were associated with a significant reduction in mortality, but also determined that no significant effect was found in prospective studies, and that the pooled

effect was attenuated when studies were analyzed according to the inclusion of important confounders in their models [187]. Another meta-analysis of 18 studies found that statins were associated with a lower risk of developing CAP and with reduced short-term mortality in patients with CAP, but that publication bias was evident in the treatment group and that there was substantial heterogeneity among the included trials [188]. Similar concerns about the low robustness and inconsistency of published trials were noted by investigators in another meta-analysis of statins in prevention of CAP [189]. Taken together, quite a few studies have now reported decreased rates of CAP and improved CAP outcomes with statins. However, given that i) virtually all of these studies have been observational and most of them retrospective; ii) many studies lack high-quality data on statin timing and adherence; and iii) many studies have not applied consistent or predefined definitions for infectious outcomes, the field currently lacks data robust enough to make confident public health recommendations. Prospective, randomized trials of statins in well-defined infections may be required to move the field forward.

Closing Remarks

The innate immune response plays a pivotal role in a wide array of inflammatory and infectious diseases in the lung and other organs. A large number of studies showing that pathogens reprogram cholesterol trafficking – often to their benefit – and, conversely, that endogenous regulators of cholesterol traffic play key roles in host defense, together provide strong evidence that cholesterol homeostasis and host defense have common evolutionary roots, and have co-evolved. Additional studies have now shown convincingly that cholesterol traffic plays a key role in lung homeostasis, including the lung's response to inflammatory and infectious particles. These studies provide new avenues for the field to harness lipid trafficking to therapeutic benefit in human lung disease. A relative latecomer to the cholesterol forum, the lung now offers new and exciting opportunities for therapeutic development. The pulmonary sciences will surely benefit from lessons learned by the cardiovascular field; perhaps the unique physiology of the lung, including its surfactant microenvironment, will offer new insights of value to the cardiovascular sciences in return.

List of abbreviations

25HC	25-hydroxycholesterol
ABCA1	ATP Binding Cassette transporter A1
ABCG1	ATP Binding Cassette transporter G1
AEC	alveolar epithelial cells
ALI	Acute lung injury
Apo	apolipoprotein
BAL	bronchoalveolar lavage
CAP	community-acquired pneumonia

CAV1(2)	caveolin-1 (caveolin-2)
FC	free cholesterol
GCSF	granulocyte colony stimulating factor
HCD/HFD	high cholesterol diet/high fat diet
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HDL	high density lipoprotein
HMGCR	hydroxymethyl-glutaryl coenzyme A reductase
IAV	influenza A virus
IL	interleukin
IFN	interferon
KC	Keratinocyte-derived chemokine
LDL	low density lipoprotein
LPS	lipopolysaccharide
LXR	Liver X Receptor
MBCD	methyl- β -cyclodextrin
MCP-1	monocyte chemotactic protein-1
MIP2	macrophage inflammatory protein 2
MMP	matrix metalloproteinase
NF-κB	nuclear factor kappa B
NPC	Niemann-Pick type C
OVA	ovalbumin
PAMP	pathogen associated molecular pattern
poly(I:C)	polyinosinic:polycytidylic acid
RCT	reverse cholesterol transport
RSV	respiratory syncytial virus
SR-BI	scavenger receptor B-I
Th	T helper
TLR	Toll-like Receptor

TNF(α)	tumor necrosis factor (alpha)
VAP	ventilator-associated pneumonia
VLDL	very low density lipoprotein
WNV	West Nile Virus

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Figure 1. Overview of cholesterol biosynthesis and trafficking

Cholesterol is metabolized to oxysterols after internalization via cell surface receptors (LDLR, scavenger receptors) or biosynthesis from acetyl-CoA via the mevalonate pathway. Oxysterols activate LXR transcription factors, and, along with cholesterol, inhibit the SREBP transcription factors. LXRs heterodimerize with RXR and induce target genes that promote cellular cholesterol efflux (e.g. ABCA1, ABCG1) and degradation of LDLR, thereby reducing intracellular cholesterol. SREBPs, activated during sterol deficit, promote sterol accumulation by inducing LDLR and many of the enzymes of the mevalonate pathway. Extracellularly, cholesterol is trafficked on lipoproteins. The liver produces and releases VLDL, which is modified into LDL, serving as the major vehicle for cholesterol delivery to peripheral tissues via its receptor, LDLR. Cellular cholesterol effluxed to lipoprotein acceptors (e.g., HDL) via ABCA1/ABCG1 is ultimately taken up by the liver via SR-BI before conversion into bile acids for release in the feces, completing the pathway of 'reverse cholesterol transport'. *ABCA*, *ATP Binding Cassette*; *HDL*, *high density lipoprotein*; *LDLR*, *low density lipoprotein receptor*; *LXR*, *liver X receptor*; *RXR*, *retinoid X receptor*; *SR-BI*, *scavenger receptor B-I*; *SREBP*, *sterol response element binding protein*; *VLDL*, *very low density lipoprotein*.

Figure 2. Integration of cholesterol metabolic and innate immune signaling in macrophages
 Recognition of pathogens and their components by their corresponding receptors initiates expression of pro-inflammatory genes (e.g. IL-6). TLR4 and its co-receptor proteins are recruited to ABC transporter-suppressible lipid rafts upon activation by LPS, inducing pro-inflammatory gene expression through IRF3 and NF- κ B, while inhibiting LXR-dependent induction of cholesterol efflux genes (ABCA1, ABCG1, apoE). Deficiency of ABCA1 or ABCG1 increases TLR4 surface expression and signaling. LXRs, activated by oxysterols originating from cholesterol synthesized in the ER via the acetyl CoA pathway or internalized by scavenger receptors, suppress NF- κ B. Some oxysterols, such as 25HC, have complex actions on host inflammatory responses, inhibiting virus and the inflammasome while augmenting AP-1-dependent gene expression. Many pathogens co-opt host raft components and can hijack raft-associated signaling pathways. Examples shown include *Listeria monocytogenes*, *Pseudomonas aeruginosa*, West Nile Virus, and HIV-1. Cholesterol depletion by statins disrupts *L. monocytogenes* infection by preventing cholesterol-dependent listeriolysin-O-mediated phagosomal escape of bacteria. *P. aeruginosa* is dependent on membrane rafts for successful propagation. WNV redistributes host cell

cholesterol to evade the immune response via inhibition of the JAK-STAT recognition system. HIV-1 requires lipid rafts for assembly and infection of target cells as well as budding, and inhibits ABCA1; however, treatment with 25HC reduces HIV-1 RNA in CD4⁺ T-cells. *ABCA*, *ATP-binding cassette*; *TLR*, *toll like receptor*; *IL*, *interleukin*; *NF-κB*, *nuclear factor kappa B*; *LPS*, *lipopolysaccharide*; *LXR*, *Liver X Receptor*; *25HC*, *25-hydroxycholesterol*, *ER*, *endoplasmic reticulum*; *WNV*, *West Nile Virus*; *HIV-1*, *Human Immunodeficiency Virus-1*; *SR-B1*, *Scavenger Receptor B-I*.

Table 1**Bacteria that Hijack Rafts**

Name	Raft-Associated Function (if known)	Raft type (when not planar lipid raft)	Ref.
<i>Campylobacter jejuni</i>	intracellular survival		[72]
<i>Legionella pneumophila</i>	intracellular survival		[73]
<i>Pseudomonas aeruginosa</i>	host response, signalling	lipid raft, caveolae, ceramide raft	[67]
<i>Brucella</i> spp.	entry/intracellular survival		[74]
<i>Escherichia coli</i>	entry/intracellular survival		[75]
<i>FimH</i> -expresion <i>E. coli</i>		caveolae	
<i>Salmonella typhimurium</i>	entry/intracellular survival		[63, 76]
<i>Shigella flexneri</i>	entry/intracellular survival		[77]
<i>Chlamydia</i> spp.	entry/intracellular survival		[78]
<i>C. trachomatis</i>		caveolae	[79]
<i>Mycobacterium</i> spp.	entry/intracellular survival		[80]
<i>M. bovis</i> ; <i>M. kansasii</i>		caveolae	
<i>Vibrio cholerae</i>	toxin binding/oligomerization		[81]
<i>Aeromonas hydrophila</i>	toxin binding/oligomerization		[82]
<i>Clostridium</i> spp.	toxin binding/oligomerization		[83]
<i>Streptococcus pyogenes</i>	toxin oligomerization		[81]
<i>Bacillus anthracis</i>	toxin oligomerization		[84]
<i>Bacillus thuringiensis</i>	toxin binding/oligomerization		[85]
<i>Helicobacter pylori</i>	toxin oligomerization/signaling		[86]
<i>Listeria monocytogenes</i>	toxin oligomerization/signaling		[87]
<i>Neisseriae gonorrhoeae</i>	Internalization	ceramide raft	[66]
<i>Staphylococcus aureus</i>	Trigger cell death	ceramide raft	[66]
<i>Pseudomonas aeruginosa</i>	Localization/Internalization	ceramide raft	[67]

Table 2

Viruses that Hijack Rafts.

Name	Viral Family	Raft-associated function (if known)	Type of raft (when not planar)	Ref.
Simian virus 40	Polyomaviridae	entry/trafficking	via caveolae	[102]
Echovirus 11	Picornaviridae	entry/trafficking		[103]
Echovirus 1	Picornaviridae	entry		[104]
Echovirus 7	Picornaviridae		caveolae	
Enterovirus 70	Picornaviridae		caveolae	
Avian sarcoma leukosis virus	Retroviridae	entry		[105]
Semliki-forest virus	Togaviridae	entry/budding		[106]
Ecotropic mouse leukemia virus	Retroviridae	entry/budding		[107]
Human T-cell leukemia virus type 1	Retroviridae	entry/budding		[108]
HIV-1	Retroviridae	entry/budding/transcytosis	also caveolae	[109]
Ebola virus	Filoviridae	entry/budding		[110]
Marburg virus	Filoviridae	entry/budding		[110]
Measles virus	Paramyxoviridae	budding		[111]
Herpes simplex virus	Herpesviridae	budding		[112]
Influenza virus	Orthomyxoviridae	budding		[90–92, 113]
Epstein-Barr virus	Herpesviridae	signaling		[114]
Respiratory syncytial virus	Paramyxoviridae	docking platform and release	also caveolae	[88–89]
Japanese encephalitis virus	Flaviviridae		caveolae	[115]
Andes virus	Bunyaviridae	internalization		[98]
Hepatitis C Virus	Flaviviridae	multiple steps		[97, 102c]
Vesicular stomatitis virus	Rhabdoviridae	budding		[116]
West nile virus	Flaviviridae			[101]
Dengue virus	Flaviviridae			[100]