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Crosstalk Between Reverse Cholesterol Transport and Innate Immunity

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Abstract

Although lipid metabolism and host defense are widely considered to be very divergent disciplines, compelling evidence suggests that host cell handling of self- and microbe-derived (e.g., lipopolysaccharide) lipids may have common evolutionary roots, and that they indeed may be inseparable processes. The innate immune response and the homeostatic network controlling cellular sterol levels are now known to reciprocally regulate one another, with important implications for several common diseases, including atherosclerosis. In the present review, we discuss recent discoveries that provide new insight into the bidirectional crosstalk between reverse cholesterol transport and innate immunity, and highlight the broader implications of these findings for therapeutic development.

Ancient Connections Between Lipid Homeostasis and Host Defense Revisited

While it has long been recognized that serum lipoproteins sequester lipopolysaccharide (LPS) and likely represent an ancient arm of the innate immune system, recent literature has led to resurgent interest in the implications of this postulate, as well as efforts to bridge the gap between the fields of lipid metabolism and host defense. Coordinate dysregulation of cholesterol trafficking and innate immunity is now recognized to play a central role in atherosclerosis and metabolic syndrome, and recent mechanistic studies suggest that lipid homeostasis and immunity may be intrinsically coupled. The present review discusses current knowledge on the trafficking pathways shared by host and microbial lipids, in mammals, as well as the mechanisms that underlie the reciprocal regulation between cholesterol trafficking and the innate immune response.

Reverse Cholesterol Transport: Old and New Views

Reverse cholesterol transport (RCT; see *Glossary*) refers to *in vivo* disposal pathways for cellular cholesterol, whereby cholesterol is homeostatically mobilized from peripheral tissues, passing through the plasma, liver, and then biliary tract before excretion in the feces (Figure 1) [1]. RCT plays a critical role in atheroprotection, and has been the topic of recent comprehensive reviews [2, 3]. It is thought that, following hydrolysis from its esterified form, free cholesterol (FC) in macrophages and other cells is initially effluxed to lipid-poor/free apolipoprotein A-I (apo-AI), via the ATP Binding Cassette transporter A1 (ABCA1).

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ApoA-I is the main protein of high density lipoprotein (HDL) particles. Nascent HDL formed by apoA-I lipidation then serves as an acceptor for additional cellular FC from the ABCG1 transporter [2]. ApoE, present in serum and macrophages, also facilitates cholesterol export. Cellular cholesterol may also be mobilized via the scavenger receptor class B type I (SR-BI) and by passive diffusion of FC and 27-OH-cholesterol, but the specific contribution of these pathways *in vivo* remains unclear. Within nascent HDL, lecithin:cholesterol acyltransferase (LCAT) esterifies FC to cholesteryl ester (CE), forming mature HDL. Cholesteryl ester transfer protein (CETP), which is present in humans but not mice, facilitates the exchange of CE in HDL particles for triglycerides (TGs) that are residing in apoB-rich lipoprotein particles such as very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low density lipoprotein (LDL). Phospholipid (PL) transfer protein (PLTP), endothelial lipase (EL), and hepatic lipase (HL) also remodel HDL. Subsequently, hepatic SR-BI and the LDL receptor take CE from HDL and cholesterol from apoB-lipoprotein particles, respectively, for transport into the liver (Figure 1). Canalicular-directed ABCG5/ABCG8 heterodimers mediate transfer of FC into the bile, and cholesterol metabolized into bile acids by CYP7A1 (classical pathway) and CYP27A1/CYP7B1 (alternative pathway) are transferred into bile by ABCB11. Notably, ABCA1, ABCG1, apoE, CETP, PLTP, ABCG5, ABCG8, and CYP7A1 (in mice) are all target genes of the oxysterol-responsive nuclear receptor Liver X Receptor (LXR), and synthetic LXR agonists enhance RCT *in vivo* [4].

In recent years, several aspects of this traditional RCT paradigm have been updated. Studies from several [5, 6] but not all [7] groups have challenged the premise of obligate passage through the biliary tract, showing that direct transintestinal cholesterol efflux (TICE) from plasma to the intestinal lumen also occurs. Obligate roles for HDL and ABCA1 have also been challenged [8]. Tissue-specific studies have shown important roles for intestinal LXR and macrophage apoE [4, 9]. LCAT has minimal effects on macrophage RCT *in vivo* [10], whereas the roles of PLTP, EL, and HL remain controversial [11, 12]. Finally, groundbreaking work has shown a role for autophagy in hydrolysis of macrophage CE [13], and for microRNA-33 in suppression of RCT [14]. As RCT is thought to reduce atherosclerosis, and potentially also inflammation and endotoxemia (as discussed below), it is presently a favored target for drug development.

Cholesterol and LPS share common trafficking and disposal pathways *in vivo*

LPS, the prototypical bacterial stimulus of the innate immune response, is a phosphorylated glycolipid structurally similar to some host-derived lipids, such as phosphatidic acid and ceramide. The common structure and amphipathic nature of anionic PLs and LPS may dictate similar requirements for binding and trafficking *in vivo*. Intriguingly, several mammalian proteins have been identified that facilitate trafficking of PL and LPS, suggesting common ancestral roots between host cell handling of self and microbial lipids (Box 1).

Complex interactions have been identified among the trafficking of PL, cholesterol, and LPS in mammals, with important implications for the innate immune response (Figure 1). LPS binding protein (LBP), CETP, and PLTP, all present in HDL particles and to varying extents in other lipoproteins, have significant homology, and have been proposed to belong to a putative family of lipid transfer and LPS binding proteins [15]. While both LBP and PLTP promote transfer of LPS to HDL and from HDL to LDL [16], only LBP facilitates binding of LPS to the LPS co-receptor CD14 on cell membranes and in plasma (i.e., soluble CD14 [sCD14]) (Figure 1). Both LBP and HDL release cell-bound LPS, and sCD14 enhances this effect, attenuating pro-inflammatory responses. However sCD14 also transfers LPS to

cellular CD14, and promotes the activation of cells not expressing CD14 [17]. While HDL is well-known to neutralize LPS, apoA-II may also enhance monocyte responses to LPS by suppressing the inhibitory activity of high concentrations of LBP [18].

LPS has common trafficking and disposal pathways with cholesterol and other host lipids. LPS competes with native apolipoproteins for binding to and cellular uptake by SR-BI/CLA-1 [19]. Conversely, ABCA1 promotes efflux of LPS, along with PL and FC, from macrophages [20]. Extracellularly, PLTP promotes binding and sequestration of LPS by HDL, and along with HDL CE, enhances its biliary-enteric elimination, after they have been taken up by SR-BI in the liver [21]. Thus, LPS rides along with cholesterol in a process of 'reverse LPS transport' [21] (Figure 1), with checkpoints in the RCT pathway thereby regulating the innate immune response through coordinate control of LPS disposal from the circulation. The common trafficking of LPS and host lipids along a single disposal pathway strongly suggests that host defense mechanisms and host lipid homeostasis are linked in mammals.

Crosstalk between cholesterol trafficking and Toll-Like Receptors

Toll like Receptors (TLRs), perhaps the best characterized pathogen recognition receptors (Box 2), have been implicated not only in antimicrobial host defense, but also in a wide variety of ostensibly non-infectious inflammatory diseases, including atherosclerosis. Recent literature has highlighted that the activation of TLRs is critically sensitive to cellular cholesterol (and thus regulated by RCT), and, conversely, that TLR activation and the acute phase response (APR) modify RCT.

Effects of TLRs and inflammation on RCT

During the APR, RCT is decreased at multiple levels, including cholesterol transporter expression, HDL quality, and HDL-cholesterol uptake and excretion by the liver (Figure 1). The relative contribution of these mechanisms may differ with the type of inflammation [22-24]. In addition to promoting cholesterol loading of macrophages (perhaps thereby supporting immune functions, as discussed below), the APR redirects HDL-cholesterol to the adrenal glands [25], supporting glucocorticoid synthesis. Thus, while the effects of the APR on RCT are pro-atherogenic, they may also support host defense functions.

Effects on cholesterol transporters—LPS downregulates macrophage ABCA1 [1, 26, 27] and ABCG1 [27], thus impairing cholesterol efflux. This and TLR3-induced cholesterol transporter downregulation occur, at least in part, through the inhibitory action of interferon regulatory factor-3 (IRF-3), at LXR binding sites in the ABCA1 promoter [26] (Figure 2). Many pro-inflammatory cytokines also downregulate ABCA1 [1, 28], although TNF α and IL-6, which are both induced in FC-loaded macrophages by ER stress [29], were shown to upregulate macrophage ABCA1 [30, 31]. Adding further complexity to the picture, LPS and zymosan have also been reported to upregulate ABCA1 [23, 32], and apoA-I itself upregulates ABCA1 through the TLR adaptor MyD88 [33], the latter suggesting a possible role for innate immune signals in cholesterol homeostasis.

Effects on HDL composition and function—In addition to reducing serum HDL, the APR causes complex changes to HDL composition [34] that in general are thought to impair its cholesterol transport and anti-inflammatory functions. In humans, decreased LCAT and CETP activity reduce formation of CE in HDL and its transfer to apoB lipoproteins. In addition, reduced levels of paraoxonase 1 (PON1) and activity of platelet-activating acyl hydrolase (PAF-AH), two HDL-associated antioxidant proteins, are thought to impair HDL antioxidant function during the APR [28, 35].

It has been shown that following LPS injection, the activity of the group IIa secretory phospholipase A2 (sPLA₂-IIa) increases, while apoA-I protein levels decrease [28]. sPLA₂-IIa also decreases HDL particle size, by enhancing the hydrolysis of PLs on the HDL surface [36]. Increases in ABCG1-dependent cholesterol efflux to HDL during the APR have nonetheless recently been attributed to enrichment in HDL PL [37]. Direct sPLA₂-IIa injection does not, however, reduce RCT *in vivo* [22].

Serum amyloid A (SAA), a major APR protein upregulated in the liver, also exerts complex effects on HDL. It is thought to displace lipid-free apoA-I from HDL, thus enhancing its clearance from the circulation [36], and reducing *in vivo* RCT [22]. SAA was also found to increase cholesterol uptake by macrophages and decrease cholesterol uptake by hepatocytes [28]. EL is also upregulated during inflammation and works in conjunction with SAA to reduce nascent HDL formation by impeding ABCA1-mediated lipidation of apoA-I [38]. Inflammation induced by zymosan, a yeast glucan, impairs RCT principally by decreasing the cholesterol acceptor ability of plasma in association with increased SAA incorporation into HDL [23]. On the other hand, SAA can itself act as an acceptor for cellular cholesterol via ABCA1- and SR-BI-dependent pathways, and has been reported to enhance HDL-induced cholesterol efflux during the APR [39].

Myeloperoxidase (MPO), a pro-oxidant protein present in neutrophils and macrophages and released during the APR, also has potent effects on HDL function. MPO binds to HDL [40], specifically targeting apoA-I for oxidative modifications that are associated with reduced cholesterol efflux and LCAT-activating function [41, 42], reduced SR-BI binding [40], and reduced RCT *in vivo* [22]. Elegant work by several groups has begun to map out the precise residues of apoA-I that are oxidized by MPO [34]. MPO has also interestingly been shown to convert HDL into a pro-inflammatory particle capable of activating NF- κ B in endothelial cells [40]. Thus, HDL is a specific target of several APR mediators, with inflammatory mediators compromising and even deranging HDLs hallmark cholesterol-mobilizing and anti-inflammatory functions.

Effects upon cholesterol transport in the liver—Two groups have recently reported that, during endotoxemia, the liver acts as the primary obstacle to RCT. McGillicuddy and colleagues reported that LPS injection reduces *in vivo* RCT, by impairing the liver-to-biliary transit of cholesterol, in association with downregulation of ABCG5, ABCG8, ABCG11, and CYP7A1 [24]. Annema et al. came to similar conclusions and found reduced expression of hepatic ABCG5, ABCG8, and ABCG11 after LPS injection, but only reduction in hepatic CYP27A1 and not CYP7A1 expression [22]. Whether these expression changes are direct or indirect in response to LPS remains unclear. By contrast, a third group reported that systemic exposure to zymosan impairs RCT primarily through reducing HDL quantity and quality [23]. Taken together, these reports indicate that microbial molecules impair *in vivo* RCT at several steps.

Effect of RCT on TLR response and inflammation

Cholesterol and its mobilization by RCT in turn regulate the innate immune response at several levels: 1) cell (plasma and endosomal) membranes; 2) intracellular signaling pathways; and 3) extracellularly, through the effects of HDL.

Cholesterol loading activates TLRs—Perhaps one of the more exciting findings of recent years in the field of innate immunity is that FC loading of macrophage membranes is sufficient to activate TLRs, perhaps due to associated changes in lipid raft microdomains. Thus, biochemical loading of macrophage plasma membrane cholesterol activates TLR4, whereas TLR3 is responsive to cholesterol loading of late endosomes [43]. Niemann Pick C

mutant cells, which have defective cholesterol trafficking to the plasma membrane associated with endosomal cholesterol overload, display constitutive overcrowding and activation of TLR4 in endosomes, suggesting that TLR4 trafficking and activity are also controlled by homeostatic membrane flow [44]. In another example of coupling between TLR traffic and membrane traffic, HDL was recently reported to induce internalization of the TLR4 adaptor TRAM to intracellular compartments (Figure 2), thus impairing subsequent TLR induction of the interferon response, although this appears to be independent of cell cholesterol stores [45].

Notably, cholesterol loading may also induce pro-inflammatory responses in cells by TLR-independent mechanisms, in part determined by the specific subcellular distribution of cholesterol. Thus, FC-loaded macrophages secrete TNF α and IL-6 through activation of the C/EBP homologous protein (CHOP) branch of the unfolded protein response, as well as other ER stress-related pathways [29]. Cholesterol crystals have also recently been shown to initiate proinflammatory signaling responses, by activating the Nlrp3 inflammasome in macrophages thus leading to activation of caspase-1 and processing of IL-1 β and IL-18 [46].

Effect of ABCA1/ABCG1 deletion on TLRs—Macrophages with cholesterol overloading, due to deficient efflux mechanisms, also display enhanced TLR responses, likely due to enhanced TLR trafficking to cholesterol-overloaded rafts. *Abca1*^{-/-} macrophages exhibit enhanced pro-inflammatory response to LPS and to specific TLR2, TLR7, and TLR9 agonists, but not TLR3 agonists, when compared to wild type (wt) macrophages [47, 48] (Figure 2). Lipid rafts of ABCA1-deficient macrophages contain increased cholesterol and increased TLR4 and TLR9 after cell exposure to the cognate ligands for these receptors [47, 48]. This suggests that ABCA1 dampens inflammation by reducing TLR trafficking to rafts through reduction of raft cholesterol. ABCA1 may also dampen LPS responses through cholesterol-independent mechanisms that involve activation of Janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3) [49]. *Abcg1*^{-/-} macrophages also have increased cell-surface display of TLR4 as well as augmented responsiveness to LPS, while macrophages with dual ABCA1/ABCG1 deficiency have even higher amplification of TLR4 responses [50], as well as enhanced TLR2/TLR4/MyD88-dependent apoptosis in response to lipid loading during efferocytosis [51]. It was also recently reported that *Abcg1*^{-/-} mice have enhanced inflammatory responses to *K. pneumoniae* infection in the lung, and improved clearance of this bacterium [52]. These observations suggest that cholesterol transporters are important regulators of the innate immune response.

Multifaceted roles for Liver X Receptors in Innate Immunity—LXR α and - β are oxysterol-activated nuclear receptors that promote RCT through induction of the transporters ABCA1, ABCG1, and other target genes [53]. LXRs have also been described to have potent anti-inflammatory function, suggesting that they may act as central integrators of sterol metabolism and immunity. For example, LXR agonists suppress proinflammatory gene induction in cells, in response to LPS and bacteria [54], and attenuate LPS-induced inflammation *in vivo*, in several organs including the lung [55]. While LXR agonists may reduce inflammatory signaling in part through the disruption of rafts in an ABC transporter-dependent fashion [56], they also suppress inflammation through transporter-independent mechanisms. For example, sumo-modified and liganded LXR is reported to transrepress LPS-induced activation of NF- κ B, in a nuclear receptor corepressor (N-CoR and SMRT) dependent mechanism [57, 58]. Recently, Mer, a receptor tyrosine kinase that facilitates efferocytosis by interacting with surface proteins on apoptotic target cells, was also identified as an LXR target gene [59]. LXR-deficient macrophages exhibit defective uptake of, and anti-inflammatory responsiveness to, apoptotic cells [59].

Interestingly, a role for LXRs in antimicrobial host defense has also been established as *Lxra*^{-/-} *Lxrb*^{-/-} mice fail to mount effective early neutrophilic, Th1, and Th17 airway responses to *Mycobacterium tuberculosis* infection, whereas treatment of wt mice with LXR agonists increases Th1/Th17 function and bacterial clearance [60]. LXR also promotes macrophage survival and pathogen clearance in the setting of *Listeria monocytogenes* infection, through induction of its target gene Apoptosis Inhibitor of Macrophages (AIM) [61]. By contrast, treatment of mice with an LXR agonist reduced neutrophil influx to the lung in response to *K. pneumoniae*, thereby impairing pulmonary clearance of this extracellular bacterium [55]. Thus, LXR appears to regulate host defense in a pathogen-dependent manner.

LXRs also have pro-inflammatory roles. Oxidized LDL and synthetic LXR ligands activate LXR and increase expression of its target gene *TLR4* in human macrophages, thereby promoting NADPH oxidase-dependent reactive oxygen species (ROS) generation [62]. Similarly, treatment of human dendritic cells with TLR4 (LPS) or TLR3 (polyI:C) ligands in the presence of LXR activation results in prolonged NF- κ B activation, augmentation of pro-inflammatory cytokine production, and an increased capacity to activate CD4⁺ T cell proliferation [63].

Taken together, as central sensors of cellular oxysterols, the LXRs likely play a pivotal role in integrating signals from sterol balance and innate immunity during metabolic stress. It is important to note in this regard that LPS itself induces cellular synthesis of oxysterol LXR ligands [64].

Multiple regulatory roles for SR-BI in innate immunity—SR-BI has recently also been shown to impact the innate immune response at several levels. Macrophages from SR-BI-null mice produce significantly higher levels of inflammatory cytokines in response to LPS than wt controls, suggesting cell-intrinsic TLR-regulatory functions of SR-BI [65]. Whether this stems from changes to lipid rafts is unclear. However, as CLA-1 and its splicing variant CLA-2 (human orthologue of the rodent SR-BII) mediate the adhesion and uptake of Gram-negative and Gram-positive bacteria [66] as well as LPS [19], clearance of microbial stimuli at the cell level may also conceivably act to dampen TLR responses. SR-BI null mice also have deficient hepatic clearance of HDL-associated LPS from serum *in vivo*, as well as deficient SR-BI-dependent cholesterol delivery from HDL to the adrenals that leads to impaired glucocorticoid synthesis during sepsis [67], which together lead to heightened inflammation and lethality. Conversely, transgenic mice overexpressing SR-BI are more resistant to septic death [65]. Taken together, SR-BI may protect against lethality in sepsis through the downregulation of macrophage inflammatory responses, enhanced clearance of LPS from the circulation, and increased synthesis of anti-inflammatory glucocorticoid.

Role of CETP and PLTP in LPS clearance and sepsis—Mice expressing human CETP have increased RCT, likely due to enhanced LDL receptor-dependent clearance of apoB lipoprotein cholesterol by the liver. They also display increased liver uptake of LPS and enhanced survival during endotoxemia [68]. Conversely, the delayed association of LPS with lipoproteins in PLTP null mice results in decreased LPS clearance, higher LPS toxicity, and a marked increase in LPS-induced mortality compared to wt controls [21]. Thus, RCT regulatory proteins in the serum coordinately control cholesterol and LPS disposal, playing an important role in the sepsis phenotype.

HDL – vehicle for lipids or mediator on the front lines of innate immunity?—Neutralization of LPS by HDL has been the subject of numerous studies. HDL binds both LPS and lipoteichoic acid, and also promotes the release of cell-bound LPS, thus reducing

cellular activation [17, 28]. Both PLs and apolipoproteins contribute to HDL-mediated neutralization of LPS [28]. Recent studies demonstrate that HDL from EL knockout mice displays enhanced LPS neutralization properties, providing further insight into its role in the metabolic regulation of the innate immune response [69]. Indeed, HDL binds and neutralizes viruses, protects against parasitic infections [28], and mediates the lysis of trypanosomes through apolipoprotein L1 (apoL1) and haptoglobin-related protein (Hpr) [70], suggesting a much broader role in host defense than just LPS neutralization. Trypanosome lytic factor, a minor subclass of HDL composed of apoL1, Hpr, and apoA-I is now known to play a critical role in protecting humans from most species of African trypanosomes, and also inhibits infection by *Leishmania* [71, 72].

In addition to its activities in the extracellular milieu, HDL also suppresses inflammatory responses through its interaction with cellular cholesterol transporters. HDL and apoA-I inhibit CD11b activation in human monocytes through cholesterol efflux-dependent raft disruption [73]. HDL also works in conjunction with ABCA1/ABCG1 to protect against oxidative stress-induced macrophage apoptosis that is mediated by TLR2/TLR4/MyD88 during efferocytosis [51]. It also inhibits hematopoietic stem cell proliferation and associated monocytosis through ABCA1/ABCG1-dependent cholesterol efflux from bone marrow stem cells [74].

It was recently reported that lipid-free apoA-I, but not HDL, activates NF- κ B and induces cytokines in macrophages through a pathway involving CD14, TLR2, TLR4, and MyD88 [33]. Consistent with these findings, and perhaps suggesting a broader class effect of the exchangeable apolipoproteins, apoC-III was also recently reported by another group to activate NF- κ B in THP-1 cells, through interactions with TLR2 [75]. HDL and apoA-I have both also been reported to induce ADAM metalloproteinase domain 17 (ADAM17)-dependent shedding of TNF α from cells, presumably in a TLR-independent manner [5]. On the other hand, apoA-I has been shown to suppress pro-inflammatory macrophage responses to LPS in part through an ABCA1- and STAT3-dependent mechanism [49, 76], and also to be anti-inflammatory in the context of autoimmunity [77, 78]. Thus, further investigation will clearly be required to fully define the pro- vs. anti-inflammatory effects of apoA-I *in vivo* under different pathophysiologic conditions. Conversely, the TLR adaptor MyD88 was found to play an important role in cholesterol efflux from macrophages *in vitro* and RCT *in vivo* [33].

We speculate that MyD88 transduces signals that support homeostatic macrophage RCT during health, and that, during the APR, altered partitioning of lipid-free apoA-I may stimulate MyD88-dependent signaling in a manner that also promotes cholesterol mobilization. Recent studies suggest that this effect does not, however, override multiple other inhibitory effects of the APR on RCT [22-24]. While it is established that MyD88-dependent inflammation contributes to atherosclerosis by recruiting monocytes to atheromas [79], future studies are warranted to discriminate whether macrophage and endothelial MyD88 serve distinct roles, in particular whether the former may counteract atherosclerosis through promoting RCT under permissive conditions.

Enhancing RCT: Broader applications on the therapeutic horizon?

Novel therapeutic approaches aimed at promoting RCT have been the topic of several recent scholarly reviews [80, 81]. Such strategies include i) direct elevation of apoA-I levels by apoA-I infusion or gene induction or derepression, ii) indirect elevation of apoA-I levels via the use of CETP inhibitors or niacin receptor agonists, iii) use of apoA-I mimetic peptides and iv) use of RCT enhancers such as LXR agonists [80]. Among several apoA-I mimetic peptides, 4F, studied in both stereoisomeric formats L-4F and D-4F, displays multiple

favorable properties. These include promotion of RCT, and anti-inflammatory and anti-oxidant activity. Of interest, L-4F also improves survival in animal models of sepsis [82] and enhances LPS neutralization by HDL [83]. D-4F reduces inflammation caused by influenza A infection [84] and attenuates airway inflammation and airway hyper-responsiveness in murine models of asthma [85]. Thus, novel RCT therapeutics have promising anti-inflammatory potential with applications that may extend well beyond the realm of atherosclerotic cardiovascular disease. Conversely, reports such as these indicate the urgent need for studies that better define the molecular and cellular mechanisms regulating RCT in organs such as the lung [84, 85]. It is intriguing to speculate that impaired RCT during sepsis [24] may have the untoward consequence of prolonging both endotoxemia and LPS association with macrophages, and, conversely, that RCT enhancers may prove useful in enhancing clearance of LPS by the liver during sepsis.

Concluding remarks

Whereas lipid homeostasis is often conceived of as a housekeeping function and innate immunity as a defense response against pathogens, an intriguing vein of literature suggests that the two processes may have evolved from a single system and that a more holistic approach to the two is thus warranted. Metabolic and inflammatory perturbations are now better understood to necessarily and intrinsically impact one another. The borderline between housekeeping and defense has been somewhat blurred by evidence that the metabolic syndrome is promoted by low-grade but detectable endotoxemia in ostensibly healthy human subjects [86] and combated by effects of TLR5 on the gut microbiome [87], and by studies showing that oxidative modification of LDL generates a particle with activity upon TLR4 [88]. Conversely, interferon-mediated downregulation of sterol biosynthesis was recently shown to be a critical component of the antiviral response, thus identifying an intrinsic role for sterol pathways in host defense [89]. Given that the sterol network and RCT may actually represent an ancient regulatory arm of the innate immune system that predates the modern epidemic of cardiovascular disease, HDL-targeted drug development for atherosclerotic cardiovascular disease will almost certainly advance insights applicable far beyond the cardiovascular system and into the much wider arenas of inflammation and infection.

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Glossary

Acute phase response (APR)	a systemic response to inflammation/injury driven by the liver and characterized by changes in plasma levels of signature proteins
ADAM metallopeptidase domain 17 (ADAM17)	a ‘shedase’ that releases membrane-anchored cytokines, cell adhesion molecules, receptors, ligands, and enzymes
Apolipoprotein A-I (apoA-I)	the major protein component of HDL
ATP binding cassette protein A1 (ABCA1)	cholesterol efflux regulatory protein involved in cellular cholesterol and phospholipid transport
ATP binding cassette, subfamily B member 11 (ABCB11)	ABC family transporter involved in export of bile salts into the biliary tract

ATP binding cassette, sub-family G member 1 (ABCG1)	ABC family transporter that regulates cellular sterol efflux to HDL
CD36 and LIMPII analog 1 (CLA-1)	human homologue of rodent SR-BI
C/EBP homologous protein (CHOP)	stress-induced protein involved in transcriptional regulation, cell cycle, and apoptosis
Cholesteryl ester transfer protein (CETP)	plasma protein that facilitates the transfer of cholesteryl esters and triglycerides between lipoproteins
Cholesterol 7α-hydroxylase (CYP7A)	rate-limiting enzyme in the biosynthesis of bile acids
Cluster of differentiation 11b (CD11b)	surface protein of leukocytes that regulates adhesion and migration
Cluster of differentiation 14 (CD14)	protein co-receptor for TLR4 and some other TLRs
Endothelial lipase (EL)	endothelial enzyme with predominant phospholipase A1 activity that acts on HDL
Endotoxemia	the presence of endotoxins in the blood, which may result in shock
Group IIa secretory phospholipase A2 (sPLA2-IIa)	acute phase phospholipase that acts on LDL and HDL
Hepatic lipase (HL)	lipase expressed in liver and adrenal glands that hydrolyzes phospholipids and triglycerides of plasma lipoproteins
High density lipoprotein (HDL)	smallest/densest plasma lipoprotein; plays critical role in RCT
Intermediate density lipoprotein (IDL)	lipoprotein formed from degradation of VLDL
Lipopolysaccharide binding protein (LBP)	APR plasma protein which regulates transfer of LPS to its receptor
Liver X Receptor (LXR)	nuclear receptor responsive to oxysterols; orchestrates RCT
Low density lipoprotein (LDL)	plasma lipoprotein that plays a central role in cholesterol delivery to peripheral cells
Myeloperoxidase (MPO)	lysosomal protein most abundantly stored in azurophilic granules of neutrophils
Paraoxonase 1 (PON1)	HDL-associated antioxidant enzyme
Phospholipid (PL)	major amphipathic lipid of cell membranes
Phospholipid transfer protein (PLTP)	protein that transfers phospholipids from triglyceride-rich lipoproteins to HDL
Platelet activating factor acetylhydrolase (PAF-AH)	a lipoprotein-associated phospholipase A2

Membrane tyrosine kinase Mer (Mer)	integral membrane protein that promotes efferocytosis and inhibits inflammatory signaling
Reverse cholesterol transport (RCT)	process whereby cholesterol of peripheral cells is ultimately disposed into the feces via passage through plasma, liver, and biliary tract
Scavenger receptor class B, type I (SR-BI)	membrane protein that promotes cellular uptake of cholesteryl esters
Serum amyloid A (SAA)	family of APR serum apolipoproteins produced by the liver
Toll like Receptor (TLR)	family of pattern recognition receptors responsive to microbial molecules
Transintestinal cholesterol efflux (TICE)	direct plasma-enteric RCT pathway that bypasses hepatobiliary system
TRIF-related adaptor molecule (TRAM)	adaptor protein that bridges TRIF adaptor to TLR4
Very Low Density Lipoprotein (VLDL)	lipoprotein made in the liver that is progressively processed into LDL

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Box 1**Lipid Homeostasis and Host Defense: two systems, or one and the same?**

Recent literature has indicated dual roles for several proteins in the binding, transfer and metabolism of both host and microbial lipids. This may reflect the structural and chemical (amphipathic) similarity between, for example, anionic phospholipids and LPS, and thus common requirements for their handling in the host. It also suggests that there may be common evolutionary roots, and perhaps intrinsic coupling, between the processes by which host cells handle fluxes in the levels of self-derived and microbial lipids.

Protein	Interactions with Host Lipids	Ref.	Interactions with Microbial Lipids	Ref.
CD14	Binds anionic PLs, modified LDL	[90]	Co-receptor for TLR2 and TLR4	[90]
TLR4	Responsive to mmLDL	[88]	LPS receptor	[91]
PLTP/LBP family	PL transfer	[92]	LPS transfer	[93]
SP-A and SP-D	PL binding	[94]	LPS binding	[95]
ABCA1	Cellular efflux of FC and PL	[1]	Cellular efflux of LPS	[20]
Scavenger Receptors	Cellular uptake of multiple lipids	[19]	Cellular uptake of LPS	[19]
AOAH	Phospholipase activity	[96]	LPS deacylation activity	[96]
Exchangeable apos	Lipid transport	[28]	LPS transport/neutralization	[17]
Annexins	PL binding	[97]	Lipid A binding/neutralization	[98]

Abbreviations: ABCA1, ATP binding cassette transporter A1; AOAH, acyloxyacyl hydrolase; Apos, apolipoproteins; FC, free cholesterol; LBP, LPS binding protein; mmLDL, minimally modified low density lipoprotein; PL, phospholipid; PLTP, PL transfer protein; SP, surfactant protein; TLR, Toll like Receptor.

Box 2**Toll Like Receptors in Host Defense and Inflammation**

Toll Like Receptors (TLRs) are a family of type I transmembrane receptors, currently thought to comprise at least 13 members in mammals, that specifically recognize a variety of microbial pathogen-associated molecular patterns (e.g., TLR4 binds LPS), and trigger host cellular responses (see recent scholarly reviews [99, 100]). TLRs are composed of three domains: an extracellular domain of leucine-rich repeat motifs thought to be involved in ligand binding; a transmembrane domain that may determine receptor localization to the plasma membrane (TLR1, TLR2, TLR4, TLR5, TLR6) or to intracellular membranes (TLR3, TLR7, TLR8, TLR9); and an intracellular tail containing a conserved Toll/interleukin-1 receptor (TIR) domain common to the IL-1 and IL-18 receptors. Upon ligand binding, TLRs signal through adaptor proteins, such as myeloid differentiation primary response protein 88 (MyD88, adaptor for all TLRs except TLR3) or TIR domain-containing adaptor inducing interferon- β (TRIF, adaptor for TLR3 and TLR4), leading to the activation of mitogen-activated protein kinases and transcription factors (e.g., nuclear factor [NF]- κ B) and induction of cytokines. TLR activity has been implicated in a wide variety of infectious and inflammatory disorders.

Figure 1. LPS and cholesterol share common trafficking and disposal pathways

Removal of cholesterol from macrophage to gut, referred to as 'reverse cholesterol transport' and how LPS trafficking integrates into it, are depicted. LBP facilitates binding of bacterial LPS to CD14 on cell membranes or in plasma (sCD14). In addition to interactions with CD14/TLR4, LPS is taken up into cells via SR-BI. HDL can release cell-bound LPS, and sCD14 enhances this release, while ABCA1 promotes additional efflux of LPS, along with FC and PL. PLTP promotes the disaggregation of LPS, allowing its binding to HDL, thereby reducing LPS interaction with cells. Following remodeling of HDL by LCAT, CETP, and PLTP, HDL cargo, including CE and LPS, are taken up by the liver in an SR-BI-dependent fashion; LDL receptor-mediated uptake of cholesterol and perhaps LPS by liver from VLDL/LDL also occurs. Cholesterol is metabolized by CYP7A1 into bile acids in the liver; free cholesterol, bile acids, and LPS are then transported into bile for elimination in the feces. A less characterized, alternate pathway for direct elimination of serum cholesterol

into the intestinal lumen, called ‘transintestinal cholesterol efflux’ (TICE), is also shown. Proteins negatively regulated by inflammation include ABC-A1/-G1/-G5/-G8, SR-BI, apoA-I, LCAT, CETP, and CYP7A1.

ABC, ATP Binding Cassette; CE, cholesteryl ester; CETP, CE transfer protein; FC, free cholesterol, HDL, high density lipoprotein; LBP, LPS binding protein; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoprotein; LPS, lipopolysaccharide; LXR, liver X receptor; PLTP, phospholipid transfer protein; RXR, retinoid X receptor; SR-BI, scavenger receptor class B type I; VLDL, very low density lipoprotein.

Figure 2. Sites of interaction between RCT and TLRs in the macrophage

ABCA1 null macrophages have an enhanced pro-inflammatory response to ligands for TLR2, TLR4, TLR7, and TLR9 compared with wt macrophages, likely reflecting increased cholesterol and TLR assembly in lipid rafts. ABCG1 null macrophages have a similar TLR-hyperresponsive phenotype. Conversely, apoA-I suppresses cytokine induction through an ABCA1-activated JAK2-STAT3 pathway and perhaps also through disrupting rafts, while HDL suppresses the type I interferon response pathway downstream of TLR4, by promoting translocation of the TLR adaptor TRAM to intracellular compartments. SR-BI also suppresses TLR4-mediated NF- κ B activation. Pathogens in turn interfere with LXR signaling by stimulating TLR3/4-dependent activation of IRF-3, which is thought to interact with the LXRE on LXR target genes, such as ABCA1 and ABCG1. HDL and apoA-I can also activate the ectodomain shedding of ADAM17 substrates, such as TNFR2, TNFR1, and TNF α , leading to their release.

ABC, ATP binding cassette; Adam, a disintegrin and metalloproteinase; HDL, high density lipoprotein; IRF, interferon regulatory factor; Jak, Janus kinase; LXR, Liver X Receptor; SR-BI, scavenger receptor class B type I; Stat3, signal transducer and activator of transcription; TLR, Toll like Receptor; TNFR, tumor necrosis factor receptor.