PERSPECTIVE

Surfactant Lipids at the Host–Environment Interface

Metabolic Sensors, Suppressors, and Effectors of Inflammatory Lung Disease

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Abstract

The lipid composition of pulmonary surfactant is unlike that of any other body fluid. This extracellular lipid reservoir is also uniquely susceptible by virtue of its direct and continuous exposure to environmental oxidants, inflammatory agents, and pathogens. Historically, the greatest attention has been focused on those biophysical features of surfactant that serve to reduce surface tension at the air–liquid interface. More recently, surfactant lipids have also been recognized as bioactive molecules that maintain immune quiescence in the lung but can also be remodeled by the inhaled environment into neolipids that mediate key roles in inflammation, immunity, and fibrosis. This review focuses on the roles in inflammatory and infectious lung disease of two classes of native surfactant lipids, glycerophospholipids and sterols, and their corresponding oxidized species, oxidized glycerophospholipids and oxysterols. We highlight evidence that surfactant composition is sensitive to circulating lipoproteins and that the lipid milieu of the alveolus should thus be recognized as susceptible to diet and common systemic metabolic disorders. We also discuss intriguing evidence suggesting that oxidized surfactant lipids may represent an evolutionary link between immunity and tissue homeostasis that arose in the primordial lung. Taken together, the emerging picture is one in which the unique environmental susceptibility of the lung, together with its unique extracellular lipid requirements, may have made this organ both an evolutionary hub and an engine for lipid-immune cross-talk.

Keywords: lung; surfactant; phospholipid; cholesterol; innate immunity

In recent years, there has been renewed interest in the susceptibility of surfactant lipids to metabolic and environmental stress and in the role that these bioactive small molecules play in immunity and inflammation. Structural features of surfactant glycerophospholipids (PLs) permit them both to interrupt pathogen interactions with the host and to "sense" and respond to environment-induced oxidative stress. In this review, after providing background on surfactant PLs and sterols and their modification during disease, we discuss emerging evidence that these lipids play a crucial role in inflammation and host defense and that their evolutionary emergence in the lung represents a crucial, intrinsic, and revealing link between metabolism and host defense.

Brief Overview of the Unique Composition and Life Cycle of Surfactant Lipid

Surfactant is ~90% lipid and 10% protein by weight (1, 2). Surfactant lipid, in turn, is ~80–85% PL, a class of lipids with a three-carbon glycerol backbone with a defining polar headgroup at the third carbon, or sn-3 position (either choline, serine, glycerol, inositol, or ethanolamine in series with a phosphate moiety), plus two acyl (fatty acid [FA]) chains at sn-1 and sn-2 (Figure 1). Within all PL headgroup classes, both acyl chains in principle can be either saturated (i.e., no double bonds, such as in palmitic acid), monounsaturated (i.e., one double bond, such as in oleic acid), or polyunsaturated (i.e., more than one double bond, such as in arachidonic acid), although the sn-1 FA is less commonly unsaturated than is the sn-2 FA. In addition to disrupting tight intermolecular packing, double bonds of unsaturated FAs make

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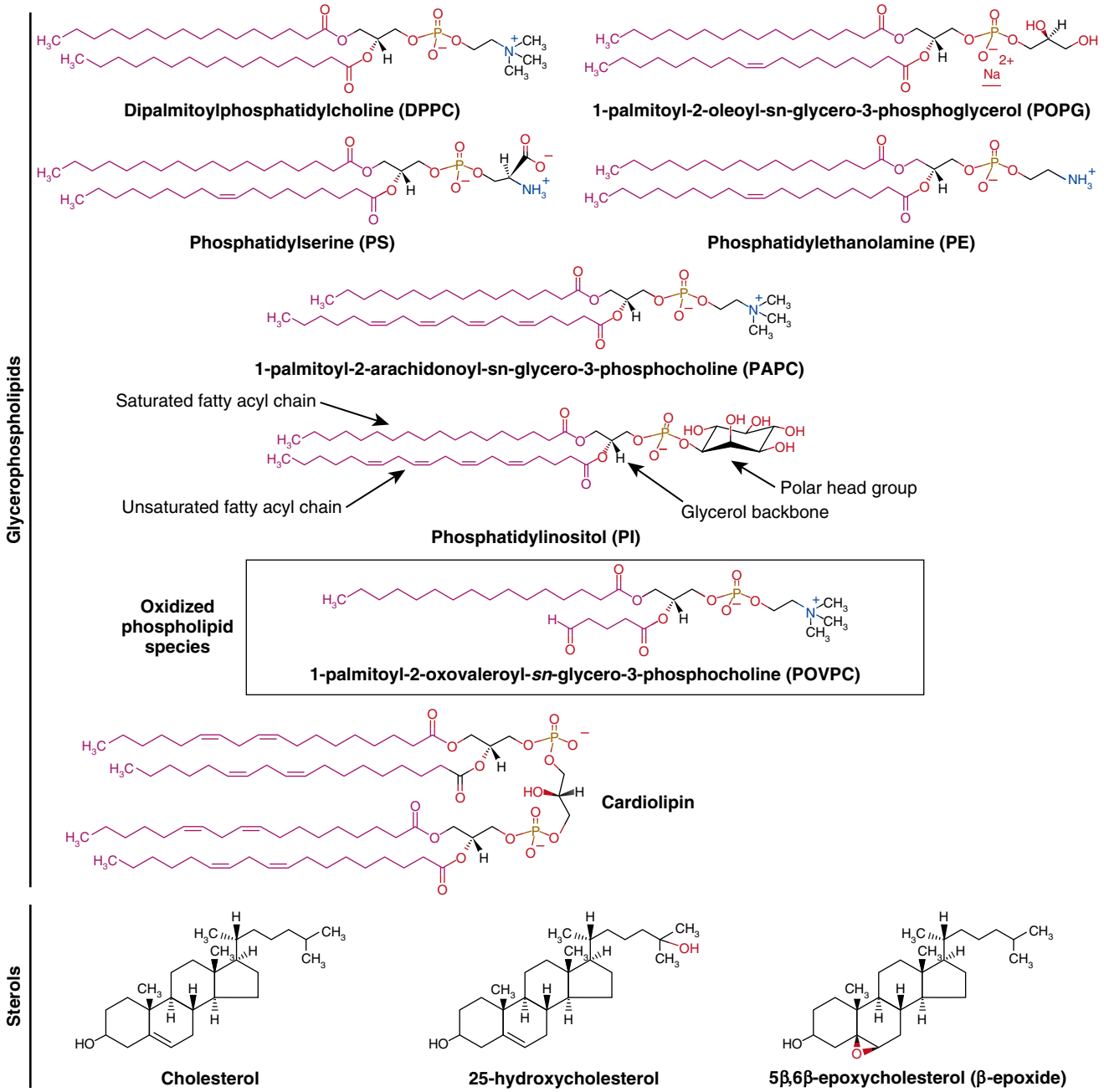

Figure 1. Representative structures of lipid species found in pulmonary surfactant. For glycerophospholipids and cardiolipin, fatty acyl side chains are depicted in *pink*, the glycerol backbone is shown in *black*, and polar head groups are located on the *far right side* of the structure. Structures shown were generated using the online structure drawing tools from LIPID MAPS (132).

them susceptible to oxidative attack, both by environmental (e.g., ozone) and enzymatic (e.g., 12/15 lipoxygenase, myeloperoxidase, NADPH oxidase) exposures. Oxidation yields complex mixtures of bioactive oxidized PL (oxPL) species, including some with fragmented FAs, such as 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine (POVPC) (Figure 1) (3). More than 20 unique native unsaturated diacyl PL species have been documented in surfactant (4), including some with highly oxidizable polyunsaturated sn-2 FAs (e.g., 1-palmitoyl-2-arachidonoyl-PC [PAPC]) (5), with unsaturated acyl chains altogether present in >30% of surfactant PL (2, 6).

Surfactant PL, estimated to be at a remarkable concentration of ~35–50 mg/ml, is a composite of phosphatidylcholine (PC, ~80%), phosphatidylglycerol (PG, ~7–15%), and small quantities (<5% each) of phosphatidylinositol (PI), phosphatidylethanolamine (PE), and phosphatidylserine (PS) (2, 7). Approximately one-half of surfactant PC, thus the most abundant surfactant PL species overall, is dipalmitoyl-PC (DPPC) (i.e., PC with two palmitic acids) (Figure 1).

It is thought that the tight intermolecular packing of DPPC, especially at end-expiration, is largely responsible for the surface tension–reducing activity of surfactant that guards against alveolar collapse (8). The hydrophobic proteins, surfactant protein (SP)-B and SP-C, aid in this function, whereas hydrophilic SP-A and SP-D are thought primarily to have host defense functions (9). Other PLs, such as PG and PI, as well as cholesterol, the major neutral lipid in surfactant (a surfactant lipid class also including small amounts of free FAs and mono-, di-, and, triglycerides), help enhance adsorption, spreading, and fluidity of the surfactant film (1). Although the PL composition of surfactant is well conserved across vertebrates, it is unlike that of either cell membranes or any other body fluid (10). For example, the concentration of even the minor surfactant PL, PG, estimated at ~3 mg/ml in humans (11), is not found at any other site in mammals, suggesting a key and perhaps unique evolutionary role for extracellular PLs in the alveolus.

The synthesis and life cycle of surfactant have been well covered in recent comprehensive reviews (8, 9, 12). In brief, surfactant PLs and SPs are synthesized in alveolar epithelial type II (ATII) cells, stored intracellularly in lamellar bodies (LBs), and secreted apically in response to catecholamines, purinoceptor agonists, and cell stretch. Extracellularly, surfactant can take multiple forms. LBs are thought to unravel and interact with SPs to form large aggregates including lattice-like tubular myelin, as well as a variety of multi- and monolayered surface films (Figure 2). Surfactant PL is regulated precisely. Approximately one-half is recycled into ATII cells for resecretion or lysosomal degradation, whereas most of the remainder is internalized and degraded by alveolar macrophages (AMs). For example, experimental depletion of AMs in rats causes surfactant PL accumulation (13). Interestingly, surfactant degradation is coupled to the maturity and immune competence of AMs. It is now well established that AM degradation of surfactant requires signaling by the maturation/differentiation cytokine, granulocyte macrophage-colony stimulating factor, and downstream activation of the transcription factors PU.1, STAT5, and peroxisome proliferator-activated receptor (PPAR)-γ (12, 14). This is best exemplified by the rare disease pulmonary alveolar proteinosis, in which disrupted granulocyte macrophage-colony stimulating factor signaling, most commonly caused by autoantibodies, is associated with surfactant accumulation and AM immune dysfunction (14). However, the intriguing finding of increased AM "foam cells" (abnormal lipid-laden AMs) in a wide range of human lung disorders (15) and rodent inhalational exposures (16, 17) suggests the provocative possibility that coordinate AM dysfunction and surfactant dysregulation may play a role in a final common pathway in the pathogenesis of common chronic lung diseases and environmental exposures.

Impact of Plasma Lipoproteins on Surfactant: The Lung as a Target Organ in Metabolic Disorders

Whereas the intra-alveolar microenvironment is often considered in isolation from the systemic circulation, extensive evidence actually suggests that surfactant lipid is sensitive to systemic metabolic status. ATII cells have long been known to bind and take up lipoproteins *in vitro*, including high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very LDL (VLDL), and to resecrete PLs bearing lipoprotein-derived FAs as well as lipoprotein-derived cholesterol (Figure 2) (18–20). ATII uptake and degradation of VLDL enhances PC synthesis through a mechanism involving activation of the rate-limiting PC-synthetic enzyme, cytidyltransferase (CCT) (19). LDL and HDL also induce PC secretion (20). Compatible with a model in which lipoproteins induce coordinated ATII release of PL and cholesterol through substrate delivery, radiolabeled lipoprotein cholesterol in an isolated perfused rat lung model was shown to be first incorporated into ATII LBs and then later released into the airspace together with PC of lipoprotein origin (21). Remarkably, intravenously injected VLDL has been shown to cross the placenta and to be incorporated as increased PC in fetal ATII cells, likely through a mechanism involving CCT activation (22). Surfactant cholesterol may be particularly dependent on systemic lipoproteins, because it has been estimated through *in vivo* labeling studies that 83% of cholesterol in the rat lung derives from uptake from the circulation (23).

Just as surfactant is sensitive to circulating lipoprotein status during health, so too is it modified during systemic metabolic disorders, although the full implications of this remain unclear. Hypertriglyceridemic apolipoprotein E–null mice, which have marked elevations in plasma VLDL, have increased alveolar PC content and increased CCT activity (22). By contrast, oxidized LDL (oxLDL), a mediator that is increased in humans with atherosclerosis (24), reduces ATII synthesis of PC, likely through promoting CCT degradation (25). oxLDL-derived oxysterols may further compromise ATII PC levels by inducing ERK-dependent inactivating phosphorylation of CCT (26) and by promoting basolateral efflux of PC via the lipid transporter ATP binding cassette (ABC)A1 (27).

Dietary perturbations have also been shown to have important effects on surfactant PLs. Rats with diet-induced hyperlipidemia have increased PG and reduced PE in surfactant associated with altered alveolar stability (28), and mice fed a high-fat diet have increased free FAs and triacylglycerol in bronchoalveolar lavage fluid (BALF) (R. S. Summer, personal communication). Ethanol ingestion also increases surfactant triacylglycerol and free FAs in BALF, likely through enhancing their synthesis by ATII cells (29), but reduces ATII synthesis of disaturated PC (30). Interestingly, dietary n-3 polyunsaturated FAs are incorporated into surfactant PLs and AM membranes in the rat (31). Furthermore, consistent with the possibility that systemic metabolic conditions may modify immune function within the alveolus via macrophage incorporation of remodeled surfactant, pulmonary surfactant from starved rats was shown to modify AM phagocytic function (32). Taken together, studies such as these indicate that the alveolar lipid microenvironment may be influenced critically by common systemic metabolic conditions. Given that HDL also serves as the major vehicle for delivery of the antioxidant vitamin E to ATII cells (33), it seems plausible that metabolic disorders with increased oxLDL and reduced/dysfunctional HDL could conspire to alter both the composition and the oxidation status of native surfactant lipids.

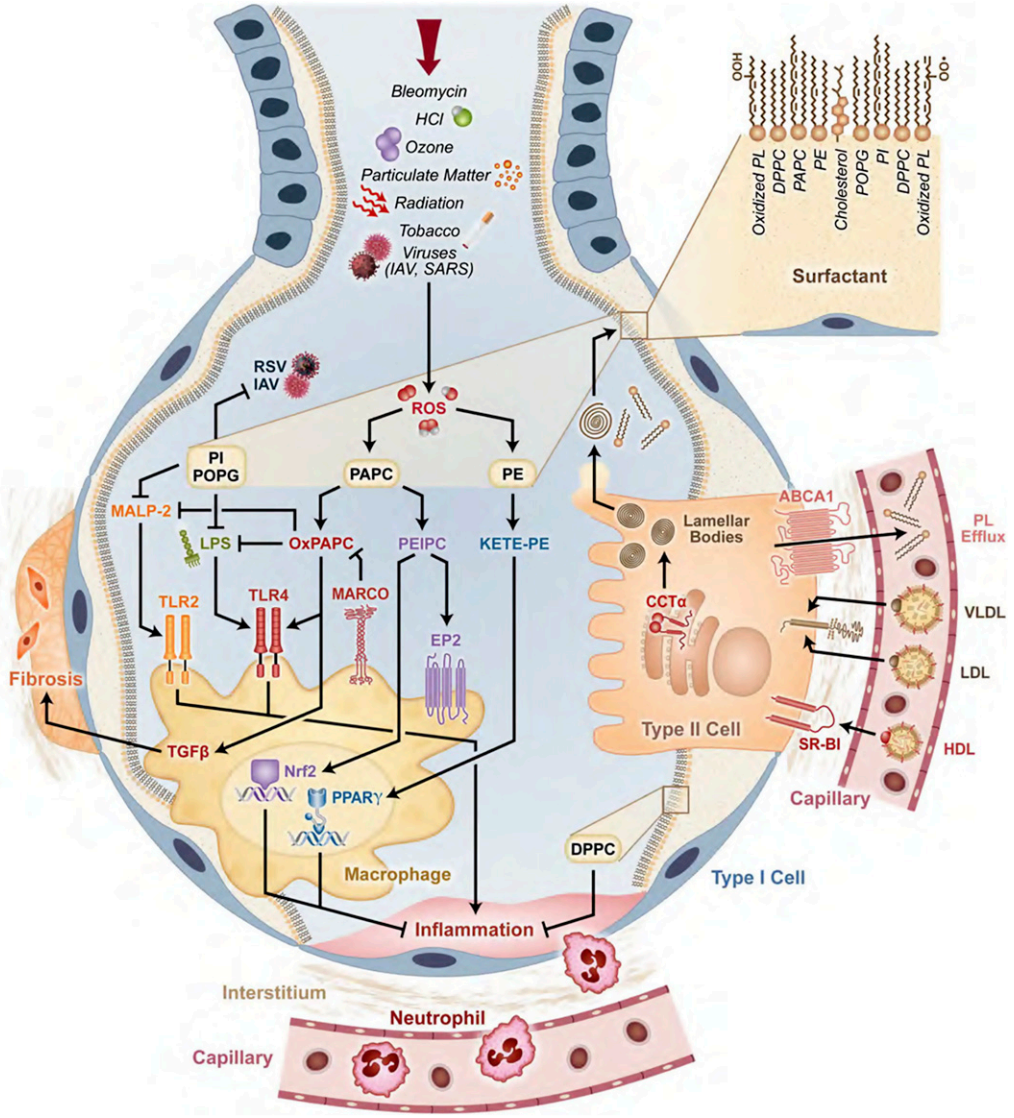

Figure 2. Roles of native and oxidized surfactant glycerophospholipids in inflammatory lung responses to the environment. The complex immune interactions that occur in the alveolus between native surfactant glycerophospholipids (PLs) and environmental agents are depicted. Surfactant PLs, including dipalmitoylphosphatidylcholine (DPPC), 1-palmitoyl-2-oleoyl-phosphatidylglycerol (POPG), phosphatidylinositol (PI), phosphatidylethanolamine (PE), and 1-palmitoyl-2-arachidonoyl-phosphatidylcholine (PAPC) are synthesized by alveolar epithelial type 2 cells and released as lamellar bodies that unravel to form the surfactant layer. Various lipids are depicted in the surfactant monolayer inset, but native surfactant is DPPC predominant. Inhaled agents and reactive oxygen species (ROS) derived from host cells oxidize PAPC and PE into oxidized PLs (oxPLs), as shown. Native PLs antagonize delivery of pathogens and pathogen-associated molecules to cellular receptors, and oxPL species act on multiple receptors on alveolar macrophages, both promoting and suppressing inflammation. Plasma lipoproteins, including high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very LDL (VLDL), deliver and receive lipids to/from alveolar epithelial cells, influencing PL synthesis. ABCA1, ATP binding cassette A1 transporter; CCT, cytidyltransferase; EP2, prostaglandin E2 receptor; IAV, influenza A virus; KETE, 15-ketoeicosatetraenoic acid; MALP-2, macrophage-activating lipopeptide-2; MARCO, macrophage receptor with collagenous structure; Nrf2, nuclear factor E2-related factor 2; oxPAPC, oxidized PAPC; PEIPC, 1-palmitoyl-2-(5,6-epoxyisoprostane E2)-sn-glycero-3-phosphocholine; PPAR, peroxisome proliferator-activated receptor; RSV, respiratory syncytial virus; SARS, severe acute respiratory syndrome; SR-BI, scavenger receptor class B member I; TGF, transforming growth factor; TLR, Toll-like receptor.

Changes in Surfactant Lipid Composition during Lung Disease

Significant changes in surfactant lipid composition have been documented in human lung diseases, in particular, acute respiratory distress syndrome (ARDS), interstitial lung disease, and pneumonia. In several studies of ARDS, decreases in PC and PG and large surfactant aggregates, and increases in protein, PI, PE, and PS have been associated with impairment in the surface tension-lowering properties of surfactant (34–36). Pathogens and pathogen-associated molecules may drive some of these changes, because *Pseudomonas aeruginosa* and adenovirus

are both reported to reduce apical PC secretion from ATII cells by promoting ABCA1-dependent basolateral PC efflux (37, 38), whereas LPS impairs DPPC synthesis by promoting degradation of acyl-coA:lysoPC acyltransferase I, an enzyme that remodels sn2-unsaturated PC into DPPC (39). In ARDS and pneumonia, the palmitic acid content of PC is indeed reduced, with associated increases in unsaturated PC species, including PAPC (35, 36), presumably rendering this PL much more susceptible to oxidation. In idiopathic pulmonary fibrosis, reduced percentages of PC and PG, increased PI, and reductions in PC palmitic acid have also been noted (34, 40). In the bleomycin rodent model of experimental lung fibrosis, significantly increased cholesterol and decreased PG have been found (16, 41).

Perhaps the most notable recent discovery in surfactant lipid dysregulation during lung disease has been the finding of abnormal accumulation during bacterial pneumonia of cardiolipin (CL), a PL with a unique tetra-acylated, di-PG-like structure that is normally restricted to the inner mitochondrial membrane (Figure 1) (42). Ordinarily present at only low levels in surfactant, CL is markedly increased in tracheal aspirates of patients with pneumonia and in BALF of mice with bacterial pneumonia. This likely occurs because of CL from dead/dying immune cells overwhelming the capacity of the CL importer, ATP8b1. Interestingly, increased CL suffices to recapitulate several hallmark features of pneumonia, including surfactant dysfunction, increased BALF protein, radiographic consolidation, and epithelial apoptosis (42). The unique structure and mitochondrial localization of CL together may explain the susceptibility of this PL, when exposed extracellularly, to targeting by autoantibodies (i.e., anticardiolipin antibodies). It is intriguing to consider the possibility that abnormal accumulation of CL during pneumonia may underlie the apparent link between respiratory infection and antiphospholipid antibody syndrome (43).

Roles of Native Surfactant Phospholipids in Inflammation and Infection

Although historically the greatest attention in surfactant science has been focused on surface tension properties (without doubt, a key evolutionary mandate that arose with tidal air breathing), a perhaps equally important requirement for successful gas exchange in the lung is the need to titrate immune responses to inhaled pathogens and pathogen-derived molecules. Over the past several years, several mechanisms have been identified by which native surfactant PLs attenuate inflammation and modify the host response to virus and bacteria (Figure 2). These findings, of course, raise the interesting yet unanswered question as to whether surfactant lipid modifications induced by diet, systemic metabolic disorders, and/or lung disease causally modify lung phenotypes in humans by modulating inflammation and/or host defense *in vivo*.

Perhaps the best-described interaction of native surfactant PLs with the innate immune response is the capacity of anionic PLs (i.e., PG, PI, PS, and CL) to interrupt the Toll-like receptor (TLR)4-mediated immune response to bacterial LPS, itself a glycolipid with an anionic PL-like core structure. This competitive antagonism almost certainly arises from structural similarity that dictates common thermodynamic requirements for protein binding and transfer through biological fluids. LPS is transferred by extracellular LPS-binding protein (LBP) to a cell-associated protein, CD14, which then relays LPS to its receptor complex, a heterodimer of TLR4 with the lipid-binding coreceptor, MD2 (44). Although LBP and CD14 are well known as LBPs to immunologists, both also competitively bind a variety of host PLs and cooperate in homeostatic PL trafficking; for example, LBP transfers PI and PS to CD14 (45). Furthermore, LBP is homologous to the lipoprotein-remodeling serum protein, phospholipid transfer protein, which is known to also transfer LPS and regulate its signaling (44), perhaps suggesting that the innate immune response and host PL homeostasis have common evolutionary roots. This intriguing possibility becomes less surprising when one realizes that certain PLs, including PG and CL, are relatively abundant in bacterial membranes, but scarce in mammalian tissues (with the interesting exception of PG in surfactant) (46).

PI and 1-palmitoyl-2-oleoyl-PG (POPG), the most prevalent PG species in human surfactant, (10) have been shown to inhibit macrophage proinflammatory responses to LPS by interfering with the LBP- CD14-TLR4/MD2 relay pathway at multiple sites. Both PLs competitively inhibit the binding of LPS to LBP and CD14 (11, 45–47), although one report suggests that PI and LPS have different binding sites on CD14 (11). POPG also competes with LPS for binding to MD-2 (11). In addition, POPG inhibits TLR2-dependent inflammatory responses, including those triggered by the respiratory pathogen *Mycoplasma pneumoniae* (48), whereas it does not inhibit responses to TLR3 (dsRNA), TLR5 (flagellin), or TLR9 (CpG DNA) ligands (11, 46). CL, in which the tetra-acylated structure is somewhat similar to that of TLR4-inhibitory tetra-acyl bacterial LPS species, also inhibits macrophage responses to LPS; this may occur through the competition for binding to LBP (47) and MD2 (49). Several reports indicate that PC is inert at inhibiting the macrophage LPS response or competing for LPS protein binding (11, 45–47), whereas others indicate that DPPC inhibits LPS-induced cytokine production by airway epithelial cells and monocytes, potentially through incorporating into the plasma membrane and affecting membrane fluidity (50–52). Intratracheal DPPC supplementation in mice attenuates lung inflammation induced by intravenous LPS (11). DPPC exerts additional antiinflammatory and protective actions, inducing prostaglandin E2 in monocytes (53), down-regulating the monocyte respiratory burst (51, 54), and protecting lung epithelial cells from invasion, lysis, and cytokine production induced by Group B *Streptococci* (55, 56).

Complex interactions have been noted between surfactant PLs and viruses. DPPC promotes adenoviral entry into epithelial cells by binding virus and serving as a vehicle for receptor-independent penetration into the cell (57). Exogenous PS also promotes cell entry by enveloped viruses, potentially through promoting fusion (58). Interestingly, PS in the poxvirus envelope promotes viral infectivity, perhaps through apoptotic cell mimicry (59), although another report indicates that PG incorporation by the viral envelope also promotes infectivity (60). On the other hand, potent antiviral activities have been shown for both POPG and PI. POPG binds respiratory syncytial virus

(RSV) and competes with RSV binding to CD14, thereby blocking viral attachment to epithelial cells, cytopathic effects, cytokine induction, and plaque formation *in vitro* (61). Remarkably, intranasal treatment of mice with POPG markedly reduces the lung viral titers and tissue injury induced by RSV (61, 62). Similar *in vitro* and *in vivo* protective effects are seen for POPG with influenza A virus (63) and for PI with RSV (64). Taken together, these findings suggest that some PLs render the airway epithelium susceptible to virus, whereas others may be protective by raising the threshold for infection.

By comparison, much less is known about the effect of surfactant lipids on bacterial infection. Free FAs in rat surfactant have been shown to kill *Pneumococcus* and other gram-positive bacteria through membranolytic detergent-like activity (65), whereas exogenous PC promotes intracellular growth of *Mycobacterium tuberculosis* in macrophages (66). Diet-induced hypercholesterolemia is also associated with compromised pulmonary host defense against *M. tuberculosis* and *Klebsiella pneumoniae* (67, 68). The interesting possibility that surfactant lipid locally programs the phenotype of alveolar phagocytes, including membrane composition and immune functions, has been suggested by some adoptive transfer and *in vitro* exposure studies (69, 70).

Oxidized Surfactant Phospholipids: Ancient Neolipids with a Key Role in Lung Disease

oxPLs, formed by chemical or enzymatic oxidation of the double bonds of unsaturated PLs such as PAPC (Figure 1), have long been studied in the cardiovascular field for their inflammatory effects, in particular, the induction of monocyte adhesion to endothelium (71). Discordant and at times seemingly contradictory effects on inflammation have been reported for oxPLs (3). This likely derives in part from the fact that methods for *in vitro* oxidation of PAPC are poorly standardized, and oxidized PAPC (oxPAPC) is actually a highly complex mixture of lipids with widely varying bioactivity (3). Investigations of defined oxPL species have benefitted from improved specificity, but likely at the expense of uncertain physiologic relevance. The remarkably pleiotropic activities of oxPLs on multiple receptors, including the platelet-activating factor receptor, prostaglandin receptors, scavenger receptors, TLRs, PPARs, and vascular endothelial growth factor receptors, as well as intracellular signals and transcription factors, were comprehensively reviewed recently (3, 72). In aggregate, these studies suggest a paradigm in which unsaturated PLs effectively serve as biosensors and ultimately as second messengers of chemical and biological oxidant stress.

Several pro-oxidant exposures have now been shown to drive the formation of oxPLs in pulmonary surfactant (Figure 2). *In vitro* exposure of human BALF to ozone directly induces multiple PC and PG oxidation products (4). *In vivo* exposure of mice to a wide variety of airway challenges, including cigarette smoke, particulate matter 2.5, acid, H1N1 influenza A virus, and H5N1 avian influenza virus also increases oxPAPC species and 1-palmitoyl-2-(9'-oxo-nonanoyl)-glycerophosphocholine (PON-GPC) in airspace fluid (73–77). Direct infection of pulmonary epithelial cells with influenza A induces their production and release of POVPC and other oxPAPC subspecies (78). Indeed, oxPAPC has been found in the lungs of patients with H5N1 avian influenza and severe acute respiratory syndrome infections, as well as in experimental animal models of anthrax, monkey pox, and *Yersinia pestis* infection, suggesting that oxPAPC may be induced as a common response to a wide range of severe pulmonary infections (76). Increased numbers of oxPAPC-laden AMs have also been documented in noninfectious human interstitial lung diseases, including desquamative interstitial pneumonitis and usual interstitial pneumonia (79). By contrast, CL and PS, but not PC, are selectively oxidized in the lungs in response to single-walled carbon nanotubes, irradiation, and hyperoxia, likely reflecting cytochrome C–driven oxidation occurring during apoptosis (80–82).

Several protective antioxidants, including urate, ascorbate, glutathione, and α- tocopherol, are present in the airway but these may be overwhelmed/depleted by acute environmental challenges, such as ozone (83). Redundant clearance mechanisms for oxPLs from the lung, including the lipid efflux transporter, ABCG1, as well as macrophage receptor with collagenous structure, a scavenger receptor, also exist (84, 85). ABCG1-dependent clearance of accumulated oxPLs from AMs is induced by HDL, an event that is likely deficient in systemic metabolic disorders with defective HDL function (86). Pulmonary expression of macrophage receptor with collagenous structure is, however, up-regulated in mice fed a high-fat diet (87).These two mechanisms, taken together, suggest that pathways for oxPL clearance from the lung may be regulated differentially by systemic oxidant burden and metabolic status.

Important proinflammatory roles have been identified recently for oxPAPC in the lung. In a landmark paper, Imai and colleagues reported that multiple chemical and infectious exposures induce NADPH oxidase–dependent production of oxPAPC in the murine lung, which then activates a pathway through TLR4 and its adaptor, TIR-domain-containing adapter-inducing interferon-β, to cytokine-dependent acute lung injury (76). A second group has shown that oxPAPC, formed in the lung during influenza A virus infection, induces TLR4-dependent, CD14-independent proinflammatory lung injury that can be suppressed by the TLR4 antagonist, eritoran (77). oxPAPC has also been reported to induce TLR2-dependent cytokines on intraperitoneal injection (88), and PON-GPC has been shown to induce cytotoxicity and cytokine production in epithelial cells in the low nanomolar range (89). Seemingly at odds with these reports are several others showing that oxPAPC attenuates the induction of proinflammatory genes by TLR4 and TLR2 ligands (3, 90–92). The former effect may arise from oxPAPC blocking interactions of LPS with LBP and CD14 (3) or MD2 (90), or by disrupting lipid raft microdomains in which TLR4 is activated (92), potentially through sphingomyelinase activation (93). Reports also differ on the question of whether oxPAPC inhibits ligand-induced activation of other TLRs, such as TLR9 (90, 91, 94). Taken together, the issue of oxPAPC activity at TLR4 may be reconciled by a model in which oxPAPC has partial agonistic activity, because it has been shown that the

LPS-inhibitory activity of oxPAPC occurs at a concentration ~10-fold lower than proinflammatory activity (95). In such a model, low-level oxPAPC might serve as a feedback brake on bacteria-induced inflammation and injury, whereas sites of localized or intense oxPAPC accumulation might drive dysregulated disease responses.

Several specific oxPL species have now been identified that are antiinflammatory and/or immunosuppressive through mechanisms other than TLR ligand antagonism (Figure 2). 1-palmitoyl-2-(5,6-epoxyisoprostane E2)-sn-glycero-3-phosphocholine (PEIPC) and 1-palmitoyl-2-(5,6-epoxyisoprostane A2)-sn-glycero-3-phosphocholine (PECPC) are oxPAPC subspecies that structurally and functionally mimic the proresolving prostaglandin 15-deoxy-D12,14-prostaglandin J2 (94). Like 15-deoxy-D12,14-prostaglandin J2, PECPC elicits antiinflammatory effects via the transcription factor nuclear factor E2 related factor 2 (94). PEIPC also induces antiinflammatory effects in macrophages, including reduced tumor necrosis factor-α and increased IL-10 production, via activation of the prostaglandin E2 receptor (96). PEIPC induced during *Mycobacterial* infection inhibits DC activation of T cells, which suggests possible adverse effects on host defense (86). PON-GPC, which is induced in the lung by cigarette smoke, is also reported to inhibit the bactericidal function of macrophages (74), whereas intratracheal delivery of oxPAPC impairs AM phagocytosis and bacterial clearance *in vivo* (75). 15-Ketoeicosatetraenoic acid–PE, a product of 15-lipoxygenase action on PE that is elevated in the BALF of patients with cystic fibrosis, and hydroperoxyeicosatetraenoic acid–PE were recently identified as antiinflammatory oxPLs that activate PPAR-γ (97). POVPC also promotes M2 polarization and TGFβ expression by AMs, inducing pulmonary fibrosis in mice (16).

Oxysterols: Bioactive Lipids with Complex Emerging Roles in Inflammatory Disease

Compared with that which is known about PLs and oxPLs, far less is known about the regulation and function of cholesterol and oxysterols in lung biology. As is the case for cholesterol in lipid raft membrane microdomains, in surfactant, cholesterol is thought to improve the fluidity/spreading of tightly packed disaturated PLs. Consequently, surfactant cholesterol levels must be regulated tightly, because excess cholesterol, such as can occur with serum leakage during acute lung injury, impairs surfactant function (98–100). Pulmonary cholesterol derives largely from plasma lipoproteins rather than *in situ* synthesis (23) and is thought to be cleared back into the plasma compartment by interactions of the lipid efflux transporters ABCG1 and ABCA1 with plasma HDL (101–103), as well as by diffusional efflux of cholestenoic acid, a hydrophilic oxysterol produced by the action of sterol-27-hydroxylase on cholesterol in AMs (104).

Cholesterol can be oxidized either on its ring structure or on its side chain (Figure 1). Analogous to the case for unsaturated PLs, ring oxidation, commonly occurring at the carbon 5–6 double bond, occurs from direct attack by reactive oxygen species. Ozone induces formation of the cytotoxic ring oxysterol 5β,6β-epoxycholesterol in pulmonary surfactant and bronchial epithelial cells (105, 106). By contrast, side-chain oxysterols are typically enzymatic products (e.g., 25-hydroxycholesterol [25HC] is produced by cholesterol-25-hydroxylase [Ch25h] action on cholesterol). Of interest, increases in both 25HC and 27HC are found in the sputum of patients with chronic obstructive pulmonary disease and are inversely related to lung function and directly related to inflammatory measures, suggesting roles in pathogenesis (107, 108).

In recent years, a profusion of papers have reported intriguing, pleiotropic biologic activities of 25HC on multiple immune receptors, with important effects on inflammation and host defense. *Ch25h* is an interferon-stimulated gene, and thus LPS, poly(I:C), viruses, and interferons all up-regulate Ch25h and increase production and release of 25HC in macrophages (109–111). Ch25h and 25HC are induced in the mouse lung in response to systemic (112) and inhaled (M. B. Fessler, personal communication) LPS challenge. Both pro- and antiinflammatory actions of 25HC have been identified. On the one hand, 25HC augments poly(I:C)-induced cytokines in airway epithelial cells via effects on nuclear factor-κB (NF-κB) (110) and amplifies TLR-induced gene induction in macrophages via enhancing recruitment of AP-1 to the promoters of proinflammatory genes (111). As a consequence, Ch25h–null mice have reduced lung injury and mortality after pulmonary challenge with influenza A (111). 25HC and some other side-chain oxysterols also promote neutrophilic inflammation by chemoattracting neutrophils via ligation of CXCR2 (113). On the other hand, 25HC has also been shown to suppress IL-1–dependent inflammation by inhibiting transcription of IL-1β and activation of IL-1β–processing inflammasome complexes (114). As a consequence, Ch25h–null mice have exacerbated mortality after systemic LPS challenge (114). 25HC also reduces IgA levels in the lung and other mucosal sites, in part by suppressing B-cell proliferation and class switch recombination (112). 7α,25-dihydroxycholesterol, a product of secondary oxidation of 25HC by the enzyme Cyp7b, was also shown recently to play a critical role in systemic humoral immunity through positioning B cells and dendritic cells in the spleen via the G-protein–coupled receptor, Ebi2 (115–119). Finally, 25HC and several other side-chain oxysterols are natural agonists of liver X receptor, a nuclear receptor that has potent antiinflammatory actions in the lung and elsewhere through the inhibition of NF-κB (120, 121). This indicates that 25HC has remarkably complex cell- and context-dependent effects on inflammation, representing a direct and intriguing link between cholesterol metabolism and immunity.

In addition to its effects on inflammation, 25HC has been shown recently to have potent antiviral effects. 25HC inhibits the growth of a broad range of enveloped viruses in cultured cells (122, 123). This may occur through blocking viral fusion (122) or alternatively through inhibiting postentry viral replication (123), potentially by activation of the integrated stress response (124). Ch25h–null mice are thus more susceptible to MHV68 lytic infection, whereas administration of 25HC to humanized mice suppresses HIV replication (122). Among naturally occurring oxysterols, 25HC is uniquely synthesized and secreted by macrophages in response to interferons and virus (123), suggesting a potentially critical role in pulmonary host defense.

Is There an Evolutionary Connection among Death, Fat, Immunity, and the Inhaled Environment?

oxPLs and oxysterols are ancient and ubiquitous modified-self autoantigens. Apoptotic cells display oxPLs on their surface that are bound by C-reactive protein and also recognized by natural (germline-encoded) antibodies (125–127). Innate B cells (i.e., B1 B cells), in particular, produce natural antibodies that recognize oxPAPC species on the surface of apoptotic cells and regulate cell corpse clearance by macrophages. Remarkably, these "T15/E06" idiotype natural antibodies also recognize phosphocholine in the capsule polysaccharide of *Streptococcus pneumoniae* and some other bacteria and are important in survival during *S. pneumoniae* infection (128). It has been proposed that these anti-oxPL B1-cell clones have been preserved through natural selection for their importance in both host defense and tissue homeostasis (126).

Interestingly, emerging evidence suggests that the lung, subject as it is to exposure to bacteria, oxPL-inducing environmental oxidants, and apoptotic leukocytes, may have a central and perhaps unique role in coordinating the regulation of lipid homeostasis and innate immunity. Abcg1-null mice have a marked accumulation of cholesterol, PL, and oxPAPC in their lungs, likely because of impaired clearance by macrophages (85, 101, 102). In parallel with this lung-selective lipid accumulation, naïve Abcg1-null mice have marked recruitment of leukocytes to their lungs (102, 129, 130). In particular, excess POVPC and other oxidized lipid species attract T15/E06-producing B1 cells to the Abcg1-null lung and drive their proliferation there, inducing a lung niche-specific autoimmune expansion that boosts systemic levels of natural anti-oxPAPC antibodies (85). Taken together, these findings suggest, intriguingly, that steady-state lipid and immune homeostasis are intrinsically linked, and that the lung, in particular, may represent a unified site for lipid-based programming and expansion of innate immune responses. T15/E06 antibody not only regulates apoptotic cell clearance (125), but has also been shown to therapeutically block oxPAPC–induced proinflammatory responses by lung macrophages (76) as well as impairment of macrophage host defense functions induced by oxPAPC and cigarette smoke–conditioned BALF (75). Given that increased native T15/E06 antibody has been detected in the lung after the induction of experimental asthma (131), it is intriguing to speculate that this natural antibody may be acutely induced in the lung in response to environment-induced oxidative stress and then serve to regulate inflammation and oxidative stress. The unique environmental susceptibility of the lung, together with its unique extracellular lipid requirements, may have made this organ both an evolutionary hub and an engine for lipid-immune cross-talk.

Conclusions

Native surfactant lipids have been recognized recently as key regulators of lung inflammation that occupy a common ground at the intersection of tissue homeostasis, host defense, and biophysics. The unsaturated bonds of PLs and cholesterol serve as biosensors of oxidative stress and endow these lipids with the flexibility to be transformed into complex, pleiotropic effectors and feedback regulators of the host response to environmental insult. Evolutionarily, the environmental susceptibility of the alveolar lipid milieu may have created selection pressure for the development of natural antibodies and other arms of the innate immune system. Although historically the lung has not been widely considered a target organ in metabolic syndromes, the alveolar microenvironment should be recognized as susceptible to dyslipidemia and other systemic disorders. It is expected that increased consideration of the lung in this light may lead to the development of novel diagnostic and therapeutic strategies in lung disease and to a better appreciation of the lung as a metabolic organ. ■

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