Phosphatidylserine in atherosclerosis

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TITLE

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Abstract

Purpose of review:

It is now widely acknowledged that phosphatidylserine (PS) is a multifunctional bioactive lipid. In this review, we focus on the function of PS in mediating cholesterol metabolism, influencing inflammatory response and regulating coagulation system, and discuss promising PS-based therapeutic approaches and detection techniques in atherosclerosis.

Recent findings:

PS has been suggested to play important roles in physiological processes, such as apoptosis, inflammation and coagulation. Recent data demonstrate atheroprotective potential of PS, reflecting its capacity to inhibit inflammation, modulate coagulation and enhance high-density lipoprotein (HDL) functionality. Furthermore, modern lipidomic approaches have enabled the investigation of PS properties relevant to the lipid-based drug delivery and development of reconstituted HDL.

Summary:

Studies of PS in relation to atherosclerosis represent an area of opportunity. Additional research elucidating mechanisms underlying experimentally observed atheroprotective effects of PS is required to fully explore therapeutic potential of this naturally occurring phospholipid in cardiovascular disease.

Keywords: Cardiovascular diseases, HDL lipoproteins, Phosphatidylserine

Abbreviations:

ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; apoA-I, apolipoprotein A-I; ER, endoplasmic reticulum; HDL, high-density lipoprotein; rHDL, reconstituted high-density lipoprotein; LDL, low-density lipoprotein; LXR, liver X receptors; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; oxLDL, oxidized low-density lipoprotein; PS, phosphatidylserine; TIM, T-cell immunoglobulin mucin.

Key points

- Phosphatidylserine of endothelial cells, leukocytes, platelets and lipid-based cargos can play important roles in atherosclerosis via resolving inflammation, expanding B1a lymphocytes, enhancing cellular cholesterol efflux capacity, inducing efferocytosis of apoptotic cells and modulating thrombolysis.

- These biological activities of phosphatidylserine are linked to intracellular signaling events stimulated directly by binding of the phosphatidylserine molecule to its receptors.

- Clinical applications of phosphatidylserine may include design of novel therapeutic agents, primarily reconstituted HDL and lipid-based drug delivery systems.

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Contents

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1. Introduction

The increasing prevalence of cardiovascular disease (CV) has stimulated an active search for novel therapeutic agents. Atherosclerosis represents a pathological process underlying the development of CV disease, characterized by fat deposits and chronic inflammation of the vessel wall.

Phosphatidylserine (PS) provides an attractive target for treatment of atherosclerosis being a natural phospholipid with multiple antiinflammatory functions which is also involved in the regulation of hemostasis and coagulation.

In cell membranes, PS is normally located in the inner leaflet of lipid bilayer, with an exceptional surface exposure during a suicide phenomenon known as apoptosis. Interestingly, PS constitutes a minor component of the surface monolayer of high-density lipoprotein (HDL), suggesting that there may be a functional requirement for PS exposure in biological systems. It is important in this context that a potent antiinflammatory functionality of PS-enriched reconstituted HDL (rHDL) has been reported (Kontush et al., 2015).

This review summarizes current knowledge in the emerging field of PS research and discusses possible functions of this lipid in the vascular biology and its implications for atherosclerosis. The roles of PS in the redistribution of plasma membrane cholesterol and HDL biogenesis will be discussed and antiinflammatory and thrombolytic mechanisms of PS effects on atherosclerotic vascular lesions reviewed. The last section describes new therapeutic and diagnostic approaches for atherosclerosis which employ lipid-based cargos enriched with PS.

2. Phosphatidylserine and cholesterol metabolism

Accumulation of cholesterol in infiltrated macrophages, which leads to the foam cell formation, is a hallmark of atherosclerosis. Molecular machinery of macrophages allows them to recognize and engulf modified apolipoprotein B (apo B)-containing lipoproteins and apoptotic bodies which both constitute two major sources of cholesterol in atherosclerotic plaques. However, due to the lack of the capacity to metabolize cholesterol, macrophages, as many other cells, express active ATP-binding cassette (ABC) cholesterol transporters, including ABCA1 and ABCG1, which enable export of the excess cholesterol to extracellular acceptors, primarily to apolipoprotein A-I (apoA-I) and HDL, in a process termed cellular

cholesterol efflux. Maintenance of cholesterol homeostasis in macrophages is mediated by intracellular sterol sensory-dependent machinery, involving liver X receptors (LXR) which regulates the expression of ABC transporters on the cell surface. Recently, an existence of a novel membrane-initiated pathway has been demonstrated which results in a rapid ABCA1 upregulation (Fond et al., 2015). This pathway relies on the PS-dependent recognition of apoptotic cells by the PS membrane receptor brain-specific angiogenesis inhibitor 1 (BAI1) and subsequent intracellular signal for engulfment and cell motility 1 (ELMO1)/Rac1 activation. Interestingly, this pathway is completely independent of the apoptotic body engulfment and, therefore, of the intracellular cholesterol sensing via LXR pathway receptors, suggesting that PS-mediated recognition of apoptotic bodies by macrophages promotes an additional signaling pathway prior to cholesterol loading to ensure optimal cholesterol hemostasis via triggering ABCA1 expression (Figure 1) (Fond et al., 2015).

ABCA1 and cholesterol efflux *per se* elicit antiinflammatory effects by virtue of reducing cell-membrane free-cholesterol and lipid-raft content. Furthermore, it is now recognized that ABCA1, beyond its activity as a lipid transporter, acts as an antiinflammatory receptor via interaction with apoA-I and subsequent activation of an antiinflammatory signaling pathway involving Janus-activated kinase-2 (JAK2)/ signal transducer and activator of transcription 3 (STAT3), which counterbalances pro-inflammatory events associated with the activation of nuclear factor-κB (NF-κB) pathway (Zhao et al., 2012). Moreover, interaction between ABCA1 and apoA-I results in the formation of HDL, which is well known to be a potent antiinflammatory lipoprotein. Taken together, PS-mediated recognition of apoptotic bodies that activates specific membrane-initiated signaling further links PS to cholesterol metabolism and lipid homeostasis (Fond et al., 2015).

Cholesterol has been identified as a crucial component of lipid rafts. However, it remains unclear how cholesterol trafficking is regulated in signal transduction hubs located in lipid rafts nanodomains.

Recently, using a newly-developed cholesterol biosensor to visualize and monitor cellular cholesterol in cytosolic leaflet in a live-cell model, a link has been found between PS and cholesterol distribution within the lipid bilayer (Maekawa and Fairn, 2015). Using PSB-2 cell line that displays very low PS synthase activities, reduction of PS content in the plasma membrane was shown to be associated with cholesterol redistribution from the inner to the exofacial leaflet of the plasma membrane (Maekawa and Fairn, 2015). Furthermore, in the lipid add-back experiments, PS supplementation largely restored cholesterol localization, while other phospholipids exhibited no effect. These data suggest that PS causes cholesterol retention in the inner leaflet of the plasma membrane, opening a therapeutic possibility to modulate pro-inflammatory signaling pathways via PS-mediated transbilayer distribution of cholesterol in lipid-raft scaffold structures.

Consistent with these observations, it has recently been shown that, depletion of PS in the inner leaflet of the plasma membrane occurs concomitantly with the depletion of cholesterol upon incubation of canine kidney cells with β-cyclodextrin, a cholesterol-depleting agent. This phenomenon was accompanied by a disruption in the organization of cholesterol and PS, both remaining in the inner leaflet of the plasma membrane (Cho et al., 2016). Although it is not yet determined how PS and cholesterol influence each other's trafficking or retention, it is of note that both are synthesized in the endoplasmic reticulum and enriched in the plasma membrane and recycling endosomes. Electrical charges resulting from the asymmetric distribution of PS and cholesterol can be involved in the control of vesiculation and subsequent trafficking (Cho et al., 2016).

Other mechanisms, including non-vesicular transport by lipid-transfer proteins, have been identified as important pathways to enrich the plasma membrane in PS and cholesterol (Chung et al., 2015; Moser von Filseck et al., 2015). In this context, the question remains as to whether PS, in addition to influencing "within-membrane" cholesterol transport, is also involved in "between-membrane" cholesterol

trafficking. Enigmatically, asymmetric enrichment of PS in plasma membranes as compared to endoplasmic reticulum (ER), where PS is synthesized, was shown to be associated with members of the lipid transport family, known as oxysterol-binding protein. Initially, these lipid-transfer proteins were found to be essential for establishing asymmetric distribution of cholesterol in Golgi versus ER membranes. Another oxysterol-binding protein subfamily involved in the lipid exchange machinery has been shown to drive asymmetric PS trafficking to plasma membranes (Moser von Filseck et al., 2015). Therefore, it is also possible that “between-membrane” transport of cholesterol is to some degree associated with PS trafficking.

Functional properties of plasma membranes are affected by modifications of their lipid composition. To date, little is known about the spatial array of membrane lipids and their potential to regulate receptor-mediated signaling. A recent study, using a direct lipid visualization method, has elegantly shown a causative association between inner plasma membrane content of PS and mislocalisation of K-Ras, a member of RasGTPase superfamily which plays crucial role in many signal transduction pathways. (Cho et al., 2016) Detailed analysis of metabolic consequences associated with acid sphingomyelinase inhibition, using its pharmacological inhibitor fendiline, revealed that the perturbation of the sphingomyelin to ceramide ratio was mechanistically associated with reduced PS and cholesterol content in the inner leaflet of the plasma membrane; such depletion of lipids was directly responsible for the mislocalisation of K-Ras isoforms. Furthermore, lipid add-back experiments revealed that there might be a distinct PS operational pool in the plasma membrane, consistent with the fact that supplementation of exogenous PS rapidly restored K-Ras assembly in the plasma membrane. However, addition of exogenous cholesterol only restored nanoclustering of one of the K-Ras isoforms, indicating the existence of a PS-dependent cholesterol pool that plays a key role in preserving cholesterol-dependent K-Ras nanoclustering in the plasma membrane. In accordance with these data, an extensive K-Ras mislocalisation has been identified in fibroblast plasma membranes in subjects with Niemann-Pick

disease whose acid sphingomyelinase is completely or partially inactivated (Cho et al., 2016). Thus, PS content in the plasma membrane contributes to K-Ras signaling by promoting plasma membrane binding and nanoclustering (Cho et al., 2016).

3. Phosphatidylserine and inflammation

It is well known that PS-mediated recognition of apoptotic bodies represents an important phagocytic response that operates by reprogramming macrophages to secrete antiinflammatory mediators such as interlukine-10 (IL-10) and transforming growth factor (TGF-β) (Huynh et al., 2002; Korns et al., 2011). Given the crucial role of PS in the apoptotic machinery, PS-containing liposomes has been used as a strategy to mimic inflammation resolving properties of apoptotic cells in order to resolve and to ultimately extinguish, inflammation. The results of these approaches has revealed several mechanisms which contribute to beneficial effects of PS-containing liposomes in suppressing inflammation, suggesting the potential of targeting macrophage-driven resolution mechanisms to attenuate atherogenesis (Fadok et al., 1998; Fitzgerald et al., 2007; Harel-Adar et al., 2011; Otsuka et al., 2007, 2004; Ramos et al., 2007).

More than ten different receptors and bridging molecules participate in the cellular PS sensing, and this complexity is expanded further by associated intracellular signaling machinery. Recent functional studies have identified a class of PS receptors that is essential for PS antiinflammatory effects (Foks et al., 2016; Hosseini et al., 2015). In essence, T-cell immunoglobulin mucin (TIM) type 1 (TIM-1) and type 4 (TIM-4) receptors function by efferocytosis of apoptotic macrophages produced by incubation with oxidized low-density lipoprotein (oxLDL) (Foks et al., 2016) as well as by expansion of B1a cells and secretion of polyreactive IgM antibodies (Hosseini et al., 2015), which in turn mediate suppressive effects of PS on inflammation and atherosclerosis (Figure 1). Indeed, supporting evidence reveals that blockade of TIM-1 and TIM-4 aggravates atherosclerosis in LDL receptor-deficient mice (Foks et al., 2016).

Investigation of detailed mechanisms of PS-mediated antiinflammatory effects represents an active research frontier; several important aspects of the process have recently emerged. Indeed, TIM-1 has been found to act as a mediator of PS liposome-induced effects on B1a cells (Foks et al., 2016). In addition, indirect B1a cell induction by PS-activated phagocytes may also take place through TIM-4 receptor (Foks et al., 2016).

Protective effects mediated by B1a cells have been attributed to the generation of natural IgM antibodies produced by these cells. These antibodies are directed against oxLDL, leucocytes, and T-cells, markedly dampening inflammation in developing atherosclerotic lesions. PS apparently plays an active role in expanding polyreactive IgM-producing B1a lymphocytes, since splenectomy abolishes the antiinflammatory and antiatherosclerotic effects of PS liposomes (Hosseini et al., 2015).

As an active metabolite of PS, lysoPS is often considered as a pro-inflammatory agent, whose production is rigorously regulated in healthy, but not in inflammatory, cells. However, lysoPS can act as an antiinflammatory and pro-resolving mediator in activated neutrophils by signaling aimed at rapid phagocytic removal of recruited neutrophils through the activation of macrophages. These findings provide further insight into potential therapeutic strategies based on the regulation of PS metabolism depending on the cell type and underlying signaling network in inflammatory diseases (Kim, 2015).

4. Phosphatidylserine and coagulation

Chronic inflammatory diseases, including atherosclerosis, are associated with a hypercoagulable and prothrombotic state. It is important in this regard that PS is widely accepted to act as a key regulator of hemostasis and thrombosis. Recently, it has been found that upon incubation of endothelial cells with uremic milieu, a characteristic of chronic kidney disease, the cells start to expose PS at the surface and form microparticles which are enriched with PS on their surfaces (Gao et al., 2015). Although it is not yet determined which specific components of uremic toxins account for a cross-talk between endothelial

cells, kidney dysfunction and increased PS exposure, in vitro experiments reveal that PS externalization in endothelial cells and peripheral blood leukocytes (He et al., 2015) provides binding sites for factor Xa and prothrombinase complexes and thereby facilitates thrombus formation (Gao et al., 2015).

On the other hand, shear stress leads to increased PS expression at the surface of platelet plasma membranes within the microenvironment of the thrombus in a process that enhances plasminogen binding and subsequent local fibrinolysis (Whyte et al., 2015). This anti-coagulant pathway contrasts with the procoagulant role of PS externalization in endothelial cells and peripheral blood leukocytes. It appears therefore that PS plays a role in maintaining a balance between pro- and anticoagulant pathways during coagulation and hemostasis. Moreover, these interesting findings suggest the importance of PS-exposing platelets as a potential target for novel thrombolytic therapy in human atherosclerotic lesions (Figure 1).

5. Outlook

5.1. Therapeutic perspectives of phosphatidylserine in atherosclerosis

Using PS as a therapeutic agent traced its conceptual roots back to the association of PS supplementation with cognitive function and memory improvement in patients with Alzheimer's disease (AD) in the late 1980s (Glade and Smith, 2015). While there is no consensus regarding the potential of PS supplementation in AD, new information on neuroprotective functions of PS has been recently published. (Donyo et al., 2016; Zhu et al., 2015).

Lipid vesicles, such as synthetic liposomes or microvesicles, are emerging therapeutic platforms for targeted delivery of multicomponent cargos. The design of an efficacious lipid vesicle composition remains a major challenge for these approaches. It is important in this regard that PS has been shown to represent an essential component for the microvesicles internalization into endothelial cells (Wei et al., 2016). Using monoclonal antibodies, PS receptor was identified as a specific cell-surface interacting

partner for such internalisation processes (Wei et al., 2016). Despite the fact that the effects of the microvesicle- or liposome-associated PS on vascular biology remain to be investigated, it is conceivable that the presence of PS on the surface of therapeutic lipid cargos can enhance endothelial uptake and consequently improve cellular delivery and efficacy.

For instance, a monoclonal antibody against PS has gained attention as a therapeutic strategy by targeting immunosuppressive functions mediated by PS (Gerber et al., 2015). Intriguingly, increased PS content in the tumor endothelial cells can potentially enable them to escape host immune cells through a mechanism similar to that known for apoptotic bodies, which includes shutting down the production of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interlukine-1β (IL-1β), or upregulating synthesis of antiinflammatory cytokines, such as IL-10 and TGF-β. Recently, a PS-targeting antibody has been successfully passed Phase 2 clinical trial in lung cancer, highlighting the potential of PS as a target for pharmacological intervention in this disease.

Size, shape and composition of therapeutic cargos may also affect subsequent signaling events through altered interactions with cell-surface receptors. This phenomenon has been particularly evidenced through immune tolerance mediated by rod-shaped poly D,L-lactide-co-glycolide (PLGA) nanoparticles (Roberts et al., 2015). Using this strategy, the enhanced antiinflammatory and effector T-cell dampening effects of PS-loaded nanorod versus liposomal PS suggested that the geometry of PS presentation itself can determine the signaling efficacy of PS-containing particles (Roberts et al., 2015). Together, these promising data highlight therapeutic potential of PS; however their relevance to atherosclerosis still remains to be established.

5.2. Phosphatidylserine and HDL functionality

HDL can be considered as an essential protective system against vascular atherosclerotic lesions. The key to understanding the role of HDL in vascular biology includes the elucidation of biomolecules

participating in the formation, remodeling and functioning of HDL. In this context, substantial compositional variations within HDL subpopulations under normal and pathological metabolic conditions have been reported, and specific roles for phospholipids and bioactive lipids carried by HDL have been proposed (Hussein et al., 2015; Kontush et al., 2015; Rached et al., 2015).

In view of the role of PS in the control of multiple biological processes, including cholesterol metabolism and inflammatory immune responses, it is of interest to study the putative role of PS as a constituent of HDL in the process of vascular homeostasis and repair. Recent studies have indicated that PS is enriched in small, dense HDL particles which display potent antiatherosclerotic activities (Camont et al., 2013). Furthermore, enrichment of rHDL with PS significantly enhanced antiatherogenic functionality of the lipoprotein (Kontush et al., 2015).

It can be speculated that PS in HDL contributes to HDL-receptor interactions. PS also appears to positively modulate HDL assembly and cholesterol loading by reducing microenvironmental pH and creating highly curved interfaces at the edge of nascent discoidal HDL (Darabi and Kontush, 2015). It is tempting to propose that selective enrichment of PS in specific HDL subpopulations can contribute to improved HDL remodeling and enhanced functionality (Darabi and Kontush, 2015).

5.3. Diagnostic applications of phosphatidylserine for atherosclerosis

Macrophage accumulation within the vascular wall has been recognized as a hallmark of atherosclerosis. Numerous studies have characterized phenotypes and subsets of human macrophages under proatherosclerotic conditions in vitro (Chinetti-Gbaguidi et al., 2014); much less work has however been devoted to studies of macrophages in atherosclerotic lesions in vivo. Labeled PS-containing liposomes have recently been developed for in vivo macrophage imaging in atheromas (Kee et al., 2015). Development of non-invasive imaging of atherosclerotic burden and inflammation using liposome-based

approaches will however require a better understanding of macrophage biology and is still in its early phase.

6. Conclusion

Present data reveal that surface PS is required for the regulation of cholesterol metabolism, inflammation and hemostasis. PS acts as a membrane ligand for specific interactions with endothelial cells, leukocytes, platelets and lipid-based cargos, modulating functional responses in target cells. All these functions are of a primary importance in the physiology of vascular system and pathogenesis of vascular dysfunction in atherosclerosis (Figure 1). As a consequence, PS-containing cargos possess a capacity to improve atherosclerosis diagnosis and treatment of this disease, providing directions for future research.

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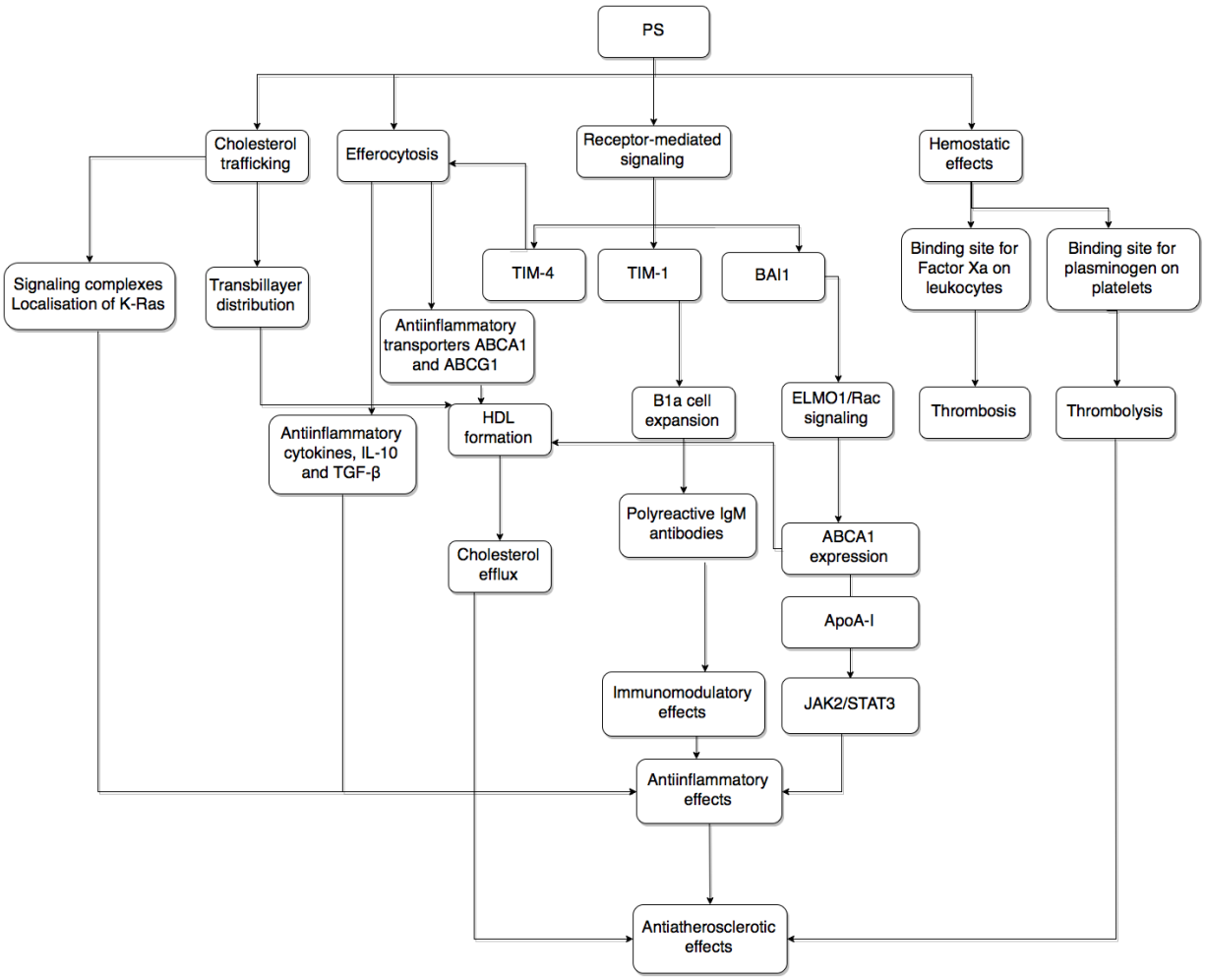

Figure 1. Potential role of phosphatidylserine (PS) in cellular and molecular processes related to the development of atherosclerosis. PS distribution in cellular membranes is tightly regulated. PS co-segregates with cholesterol in the inner leaflet of the plasma membrane. In turn, PS-mediated transbilayer distribution of cholesterol affects lipid raft-mediated signaling, membrane localisation of signaling complexes, including K-Ras, and cholesterol availability for HDL formation and HDL-mediated cellular lipid efflux. Activation of engulfment and cell motility 1 (ELMO1)/Rac1 signaling pathway via the PS membrane receptor brain-specific angiogenesis inhibitor 1 (BAI1) leads to a rapid ABCA1 upregulation on macrophages. The increase in ABCA1 not only promote cholesterol efflux capacity but can also activate an antiinflammatory signaling pathway via Janus-activated kinase-2 (JAK2)/ signal

transducer and activator of transcription 3 (STAT3). PS-containing liposomes expedite expansion of B1a cells, secreting polyreactive IgM antibodies in a T-cell immunoglobulin mucin (TIM) type 1 (TIM-1) receptor-dependent manner. The liposomes can also activate phagocytes through TIM-4 receptor, promoting efferocytosis of apoptotic bodies. PS externalization in endothelial cells and peripheral blood leukocytes can promote thrombus formation by factor Xa binding, while activating local fibrinolysis via binding plasminogen at the surface of platelet plasma membranes within the microenvironment of the thrombus, consistent with a dual regulatory role of(Kerner et al., 2005) PS in hemostasis.

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* of special interest

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