Arch. Immunol. Ther. Exp. (2011) 59:195–201
DOI 10.1007/s00005-011-0123-4

REVIEW

Phosphatidylserine-Containing Liposomes: Potential Pharmacological Interventions Against Inflammatory and Immune Diseases Through the Production of Prostaglandin E_2 After Uptake by Myeloid Derived Phagocytes

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Received: 28 September 2010 / Accepted: 5 November 2010 / Published online: 10 April 2011

Abstract Phosphatidylserine (PS), which is normally located on the inner leaflet of the plasma membrane, translocates to the outer leaflet at the early stage of apoptosis. The PS externalization provides a signal for phagocytes to initiate uptake of apoptotic cells. After phagocytosis of apoptotic cells, phagocytes induce the secretion of anti-inflammatory mediators including prostaglandin E_2 (PGE_2). PS-containing liposomes (PSLs) can mimic the effects of apoptotic cells on phagocytes to induce the secretion of PGE_2. PSLs induce the PGE_2 secretion from microglia without induction of either cyclooxygenase (COX)-2 or microsomal prostaglandin E synthase (mPGES)-1. PSLs are found to rather utilize COX-1/mPGES-2 system to produce PGE_2 secretion and then shift microglia and macrophages from pro- to anti-inflammatory phenotype by an autocrine action of PGE_2. Moreover, PSLs inhibit the maturation of dendritic cells and osteoclast precursors. Therefore, PSLs will be potential pharmacological interventions for inflammatory and immune diseases through feedback mechanism utilizing PGE_2.

Keywords Phosphatidylserine (PS) · PS-containing liposomes · Phagocytes · PGE_2

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Introduction

Myeloid derived mononuclear cells in mammalian body, including monocytes in blood, macrophages in spleen and microglia in brain, can phagocytoze apoptotic cells and necrotic cell debris in the tissues, are known as phagocytes (Athanasou and Quinn 1990). Besides these professional phagocytes, non- professional phagocytes such as dendritic cells (DCs), the myeloid-derived professional antigen-presenting cells, can phagocytoze apoptotic cells (Iyoda et al. 2002; Steinman et al. 2000). Moreover, the myeloid derived multinucleated cells, osteoclasts, which generally remove mineralized matrix resulting in bone resorption, can also phagocytoze apoptotic bone cells (Boabaid et al. 2001; Cerri et al. 2003).

Increasing evidences show that recognition and uptake of apoptotic cells by phagocytes are initiated by the exposure of phosphatidylserine (PS) on the cell membrane, and PS-dependent apoptotic cell phagocytosis lead macrophages to anti-inflammatory phenotype during immune response (Fadok et al. 1998; Hoffmann et al. 2001; Huynh et al. 2002). Prostaglandin E_2 (PGE_2), one of powerful short-lived lipid, can shift the activated phagocytes to anti-inflammatory phenotypes, in turn to cause myeloid derived phagocytes play anti-inflammation and immune suppression. On the other hand, PS-containing liposomes (PSLs) can mimic the effects of apoptotic cells on the myeloid derived phagocytes including macrophages, microglia and DCs to shift them from pro- to anti-inflammatory phenotype through the secretion of anti-inflammatory mediators including PGE_2 (Otsuka et al. 2004; Shi et al. 2007; Wu and Nakanishi 2010; Zhang et al. 2006).

This review highlights how PGE_2 modulates the differentiation and functions of phagocytes within immune responses, and discusses the potential therapeutic

usefulness of PSLs on inflammatory and immune diseases through increase PGE_2 production.

Effects of PSLs on Microglia/Macrophages

It is well accepted that microglia are derived from myeloid origin and are responsible for the innate immune response in the brain central nervous system (CNS) (Perry and Gordon 1988). In a response to brain injuries or immunological stimuli, microglia usually become readily activated to release a variety of soluble factors, which are pro-inflammatory in nature and potentially cytotoxic (Kreutzberg 1996; Liu and Hong 2003). PGE_2, one of the major prostaglandins produced in the CNS, potently and selectively inhibits the tumor necrosis factor (TNF)-α production, but enhances the interleukin (IL)-10 production in lipopolysaccharide (LPS)-stimulated primary microglia (Aloisi et al. 1999; Petrova et al. 1999). PGE_2 also reduces the release of IL-18 and nitric oxide from microglia (Kim et al. 2002; Suk et al. 2001). The inhibitory effect of PGE_2 on LPS-induced IL-18 production depends on the suppression of nuclear factor (NF)-κB activation (Suk et al. 2001). Agents that increase cAMP levels mimic the action of PGE_2 on cytokine secretion, indicating the involvement of cAMP-coupled prostaglandin receptors (EP2 and/or EP4 receptors). In the in vivo experiments, a high concentration of PGE_2 reduces the activity of microglia in the damaged CNS (Aloisi et al. 1999). Therefore, exogenous PGE_2 can protect neuronal damages through modulating pro- and anti-inflammatory mediators secreted from microglia.

There is increasing evidence that PGE_2 negatively regulates the production of pro-inflammatory mediators including TNF-α by activated macrophages (Chae et al. 2008; Fennekohl et al. 2002; Rouzer et al. 2004; van der Pouw Kraan et al. 1995). Endogenously produced PGE_2 limits the TNF-α production by LPS stimulated macrophages and peripheral blood monocytes in an autocrine fashion (Chae et al. 2008; Dieter et al. 1999; Fennekohl et al. 2002; Rouzer et al. 2004). The inhibitory effect of PGE_2 on LPS-induced TNF-α production by macrophages is mediated by an increased level of intracellular cAMP, which selectively activates protein kinase A type I to inhibit TNF-α gene transcription (Stafford and Marnett 2008). In addition to the inhibitory role on TNF-α production, PGE_2 also attenuates LPS-induced expression of chemokines including monocyte chemoattractant protein-1, IL-8, macrophage inflammatory protein (MIP)-1α and -1β, and interferon (IFN)-inducible protein-10 (Takayama et al. 2002). A selective EP4 receptor antagonist completely reversed PGE_2-induced suppression of the chemokine production (Takayama et al. 2002). Moreover, EP4RAG, a selective EP4 receptor agonist, suppresses pro-inflammatory cytokines and chemokines by inhibiting the activation NF-κB (Ogawa et al. 2009). Therefore, it is considerable that the inhibitory effect of PGE_2 on the production of pro-inflammatory mediators by macrophages is mediated by the suppression of NF-κB activation through EP4 receptors (Ogawa et al. 2009; Takayama et al. 2002).

On the other hand, PGE_2 positively regulates the production of anti-inflammatory cytokines by activated macrophages. PGE_2 enhances the IL-10 production by cultured macrophages dependent on cAMP-inducing capacity (van der Pouw Kraan et al. 1995). In the in vivo experiments, increased PGE_2 in mycobacterium microti-infected mouse closely linked with the increased IL-10 production by peritoneal macrophages, because indomethacin, a preferential cyclooxygenase (COX)-1 inhibitor, significantly decreases the IL-10 production. Furthermore, mycobacterial products induced the PGE_2-releasing from mouse spleen macrophages is associated with the expression of COX-2, because NS-398, a selective COX-2 inhibitor, reduces the IL-10 production (Mittal et al. 2001; Shibata et al. 2005). A more recent report shows that PGE_2 is responsible for the increased production of IL-10 by LPS-stimulated peritoneal macrophages in pristane-induced lupus mice, because indomethacin significantly inhibits the LPS-induced production of IL-10 by the macrophages (Chae et al. 2008).

PSLs can mimic the effects of apoptotic cells on microglia and macrophages. A series of studies has shown that PSLs strongly reduce the release of pro-inflammatory mediators including nitric oxide, IL-1β and TNF-α by LPS-activated microglia (De et al. 2002) and macrophages (Wu et al., unpublished data). In our recent study, microglia rapidly produce PGE_2 after treatment with PSLs (Zhang et al. 2006). High density lipoprotein, a specific ligand for class B scavenger receptor type I (SR-BI), significantly suppressed both phagocytosis of PSLs and the subsequent PGE_2 production by microglia though the COX-1/microsomal prostaglandin E synthase-2 (mPGES-2) pathway (Fig. 1).

Effects of PSLs on DCs

DCs play major roles in induction of primary immune responses and tolerance (Banchereau and Steinman 1998). Activated DCs can produce large amounts of PGE_2 to modulate their functions in an autocrine manner (Harizi et al. 2002). EP2 and EP4 receptors are efficient for mediating PGE_2-induced modulation of activated DC functions, because the expression of EP2 and EP4 receptors are enhanced by LPS in a dose-dependent manner (Harizi et al. 2003). LPS-induced PGE_2 synthesis by DCs depends on the activated p38 MAPK-induced COX-2 expression, because

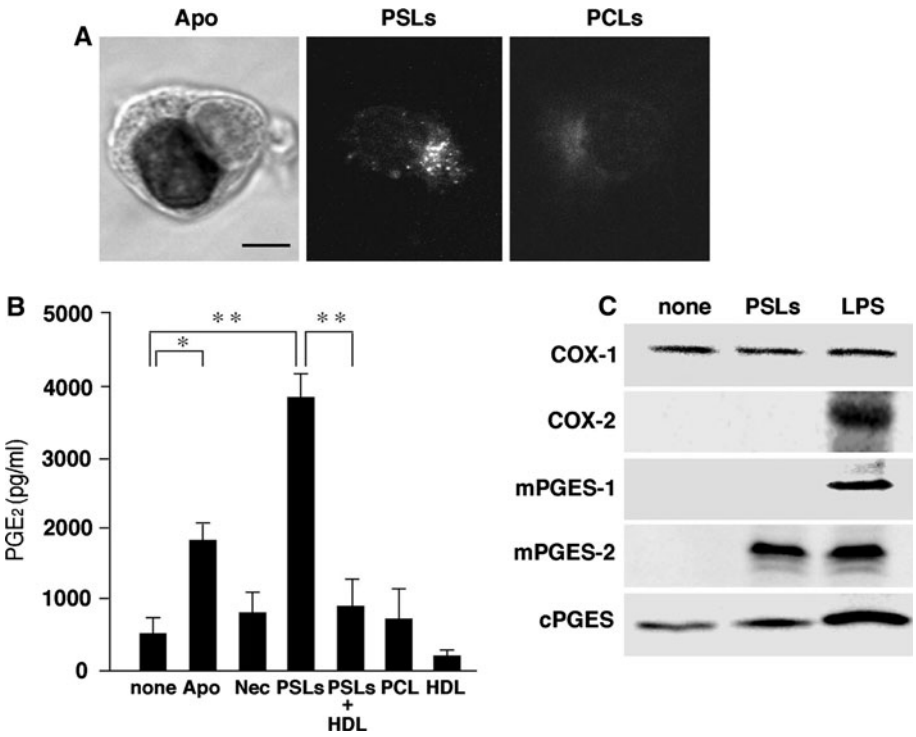

Fig. 1 Production of PGE_2 after phagocytosis of apoptotic neurons and PSLs by microglia. **a** Phagocytosis of TUNEL-positive apoptotic neuron (*Apo*) and 4-nitrobenz-2-oxa-1,3-diazole (*NBD*)-labeled PSLs but not PC liposomes (*PCLs*) by primary cultured microglia. *Scale bar* 10 μm. **b** Production of PGE_2 after phagocytosis of Apo and PSLs but not necrotic neurons (*Nec*) or PCLs by primary cultured microglia. The amount of PGE_2 in the culture medium were determined after 3 h treatment with Apo, Nec, PSLs (100 μm) or PCLs (100 μm) in the presence or absence of high density lipoprotein (HDL, 1 mg/ml). Apo and Nec were induced by treatment with sodium nitroproside (100 μm) and boiling, respectively. *None* non-treatment. Each *column* and *bar* represent the mean ± SEM of three experiments. *Asterisks* indicate significanct difference between the values ($*p < 0.05$; $**p < 0.01$, Mann–Whitney *U* test). **c** Differential requirement of COX and PGES isoforms for PGE_2 production by microglia after treatment with PSLs and LPS. Microglial protein extracts after 3 h treatment with PSLs (100 μm) or LPS (10 ng/ml) were subjected to immunoblot analyses. *None* non-treatment

p38 MAPK inhibitors block the LPS-induced COX-2 expression and PGE_2 release (Norgauer et al. 2003). PGE_2 inhibits TNF-α release from LPS stimulated murine bone marrow (BM)-derived DCs (BM-DCs) (Vassiliou et al. 2003). Furthermore, exogenous PGE_2 inhibits CCL3 (MIP-1α) and CCL4 (MIP-1β) expression and release from LPS-stimulated DCs through EP2 and EP4 receptors (Jing et al. 2003). On the other hand, PGE_2 enhances the IL-10 production by BM-DCs. Furthermore, a selective inhibitor of EP2 receptor agonist, butaprost enhances the IL-10 production from DCs (Harizi et al. 2003). The increased IL-10 then participates in down-regulating the TNF-α production from BM-DCs (Harizi and Norbert 2004). Moreover, a recent report shows that PGE_2 released from mesenchymal stem cells (MSCs) inhibit DCs maturation and function. NS-398, a selective inhibitor of COX-2, restores the MSCs inhibitory roles on DC differentiation and function (Spaggiari et al. 2009). Therefore, PGE_2 plays crucial roles in determining the DCs differentiation and functions.

PSLs inhibit the DC maturation to modulate adaptive immune responses. PSLs decreased the up-regulation of HLA-ABC, HLA-DR, CD80, CD86, CD40, and CD83, as well as the production of IL-12p70 by LPS-stimulated human DCs. After exposure to PSLs, DCs diminish capacity to stimulate allogeneic T cell proliferation and to activate IFN-γ-producing $CD4^+$ T cells (Chen et al. 2004). Recently, in vivo study also demonstrates that PSLs reduce the expression of MHC II, CD80, CD86 and CD40 by DCs. Furthermore, PSLs inhibit the IL-12 production but increase the IL-10 production by DCs prepared from 1-chloro-2,4-dinitrobenze (DNCB) challenged mice. PSLs also suppress the IFN-γ production by DCs prepared from DNCB-challenged mice (Shi et al. 2007). Therefore, the effects of PSLs on DCs may be mediated by the PGE_2 production. However, little information is available about the involvement of PGE_2 in the effects of PSLs on DCs,

Effects of PSLs on Osteoclasts

Osteoclasts are differentiated from the myeloid precursors (Miyamoto et al. 2001; Teitelbaum and Ross 2003). Although PGE_2 is believed to stimulate the murine osteoclast differentiation at a relatively high concentration

(Hurley et al. 1998; Okada et al. 2000), PGE_2 has been proved to strongly inhibit the human osteoclast differentiation (Take et al. 2005). PGE_2 strongly inhibits receptor activated NF-κB ligand (RANKL)-induced human osteoclast differentiation in the $CD14^+$ human peripheral blood mononuclear cells which expressed EP2 and EP4 receptors. H-89 can reverse the inhibitory effect of PGE_2 on $CD14^+$ cell cultures. These results indicate that the inhibitory effect of PGE_2 on the human osteoclast differentiation is mediated by EP2/EP4 receptors. More interestingly, the conditioned medium of $CD14^+$ cells pretreated with PGE_2 inhibits RANKL-induced osteoclast differentiation in both human $CD14^+$ cell cultures and murine macrophage cultures. Our recently findings show that a low concentration of PGE_2 (500 pg/ml) significantly inhibits osteoclast differentiation in rat culture systems (Wu et al. 2010) which similar to the previous data in rat culture (Itonaga et al. 1999; Roux et al. 1997). The inhibitory effect of PGE_2 on osteoclast differentiation is significantly antagonized by SQ22536, an adenylate cyclase inhibitor, thus suggesting the involvement of EP2 and/or EP4 receptors that are coupled to adenylate cyclase in the effect of PGE_2 (Take et al. 2005; Tanaka et al. 2004). It is well known that the receptor RANK/RANKL pathway plays essential roles in differentiation and the fusion of the precursors into mature osteoclasts (Kong et al. 1999; Li et al. 2000). Our recently study shows that PGE_2 suppresses RANKL expression in the BM cells during the early stage of osteoclast differentiation in rat culture system. Furthermore, PGE_2 suppresses the ICAM-1 expression in osteoclast precursors, an important adhesion molecular for the osteoclast precursor fusion (Wu et al. 2010). Because osteoclasts precursors have both EP2 and EP4 receptors (Kobayashi et al. 2005), PGE_2 may inhibit osteoclast differentiation partly in an autocrine manner.

We have recently found that PSLs significantly inhibit the formation of mature osteoclasts but not that of osteoclast precursors. PSLs can be phagocytosed to enhance the secretion of PGE_2 quickly by the osteoclast precursors. The inhibitory effect of PSLs on osteoclast differentiation is significantly antagonized by SQ22536 in rat culture systems. It is believed that the inhibitory effects of PSLs on the osteoclast differentiation may partly depend on the PGE_2 secretion (Wu et al. 2010; Fig. 2).

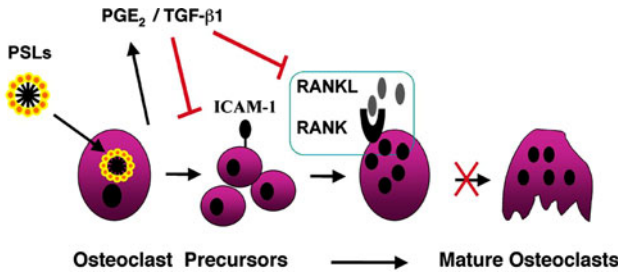

Fig. 2 Schematic of the PSLs inhibitory effects on osteoclast differentiation. PSLs can phagocytosed by osteoclast precursors, to promote their soluble factors production, including PGE_2. The PSLs increased PGE_2 down-regulates ICAM-1 and RANKL expression in turn to inhibit osteoclast differentiation

Effects of PSLs on Osteoblast

Besides the effects on the myeloid derived phagocytes, PGE_2 also works on MSC-derived non-professional phagocytes, such as osteoblasts. Previous in vitro studies documented that PGE_2 stimulated both early-stage markers of osteoblasts, including alkaline phosphatase (ALP) and collagen expression (Hakeda et al. 1985) and late-stage markers, osteocalcin (OCN) expression and mineralization (Conconi et al. 2002; Flanagan and Chambers 1992; Nagata et al. 1994), suggesting that PGE_2 stimulates osteoblasts differentiation. More recently in vivo studies show that PGE_2 increases the bone mass in both rats (Tian et al. 2008) and mice (Gao et al. 2009). Therefore, PGE_2 has widely accepted to be a potent stimulator on bone formation. PGE_2 promotes osteoblasts differentiation probably through EP4 receptors. Because ONO-4819, a selective EP4 receptor agonist, induces the osteoblast differentiation in BM cell culture, and increases the density of osteoblasts lining the bone of normal rats and restored the volume of cancellous bone in ovariectomized rats (Yoshida et al. 2002). Furthermore, AH-23848B, a selective inhibitor for EP4 receptors, abrogates the PGE_2-induced osteoblast formation promoting effects (Fujino et al. 2005). Because the PGE_2-induced elevation of cAMP through EP4 receptors results in increase of protein kinase A (PKA) activity in various cell types, cAMP-dependent PKA activation plays a key role in PGE_2-associated effects in osteoblasts (Wu et al. 2007).

Our ongoing studies show that PSLs enhances the expression of ALP and OCN, and promotes mineralization in the rat primary osteoblats. Furthermore, PSLs increase the expression of Runt-related factor-2, one of the master regulators of osteogenesis (Ma et al., unpublished data). The effects of PSLs on osteoblasts maturation are partly mediated by the PGE_2, because PSLs increase PGE_2 production in whole BM cells as well as osteoclast precursors (Wu et al. 2010). Therefore, PSLs may not only regulate the differentiation and function of myeloid derived phagocytes, but also act on the MSC derived non-professional phagocytes. Several types of drugs currently are used for the treatment of bone loss by either inhibiting differentiation functions of osteoclasts or activating osteoblasts. However, none of these drugs are able to restore the balance between bone formation by osteoblasts and bone resorption by osteoclasts. PSLs appear to inhibit

osteoclastogenesis and induce osteoblastogenesis to integrate the two actions temporarily and spatially in bone remodeling.

PSL-Induced Novel PGE_2 Production Pathway

It is described the contribution of PSLs in the PGE_2 production by phagocytes during immune response. The preceding paragraphs will summarize and discuss the pathway for PSL-induced PGE_2 production.

Biosynthesis of PGE_2 is accomplished in a metabolic cascade starting from its precursor fatty acid, arachidonic acid (AA). AA is present in membrane phospholipids and released by the hydrolytic action of phospholipase A_2. Released AA is metabolized to the unstable intermediate prostanoids by COX, also called PGH_2 synthase. COX has bifunctional catalytic properties. It oxygenates and cyclizes AA to form PGG_2 via its cyclooxygenase function, and this is followed by the reduction of PGG_2 to PGH_2 via its peroxidase function. PGH_2 is then converted to PGE_2 by the terminal synthases. PGE_2 synthesis is mainly determined by the availability of the substrate AA and the COX activity. Since AA in total cellular fatty acid or in phospholipids fraction has been shown either to remain unchanged or to decrease with age, the rate-limiting enzyme COX became the focus of our study. There are different isoenzymes of COX, COX-1, a constitutive form, and COX-2, an inducible form. COX-1 is constitutively expressed and is believed to be responsible for producing prostanoids to maintain physiological functions such as gastric protection and renal function. In contrast, COX-2 is regulated by growth factors, tumor promoters, cytokines, mitogens, glucocorticoids, and bacterial endotoxin, and is implicated in inflammatory responses and pathological changes in numerous disorders. PGES is not considered a rate-limiting factor in PGE_2 production, although recent publications suggest that it couples to COX and may participate in regulation of PGE_2 synthesis. The study shows that activity and expression of mPGES-1 in peritoneal macrophages increase after pro-inflammatory stimuli. mPGES-1 is shown to be functionally coupled with COX-2 as compared to COX-1(Murakami et al. 2000).

In contrast, our study shows that microglia rapidly produce PGE_2 after treatment with PSLs. The PSL-induced PGE_2 production does not depend on COX-2, because neither COX-2 nor mPGES-1 is induced after treatment with PSLs. Instead, terminal PGESs, mPGES-2 and cytosolic PGES (cPGES), that are functionally coupled with COX-1 (Murakami et al. 2003) are markedly increased. Furthermore, PSL-induced PGE_2 production by microglia is significantly suppressed by indomethacin but not by NS-398. Therefore, we believed that COX-1 and its functionally coupled up-regulated mPGES-2 and cPGES, play key roles in PSL-induced PGE_2 production by phagocytes on turn to inhibit their activation (Wu and Nakanishi 2010; Fig. 3). Moreover, we also demonstrated that phagocytosis of PSLs promote the PGE_2 production by transducing intracellular signals including extracellular signal-regulated kinase 1/2 but not p38 MAPK.

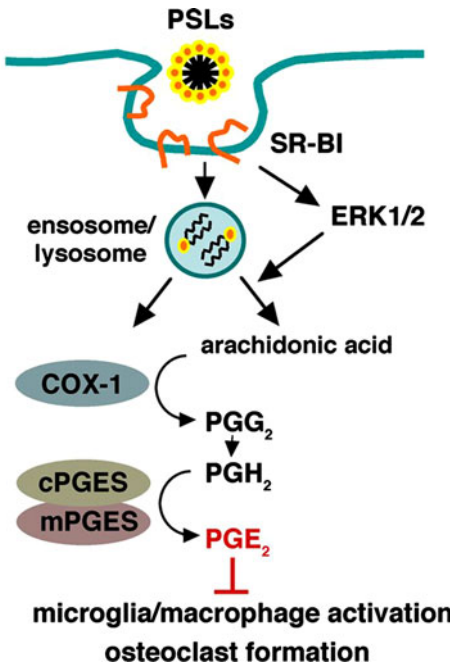

Fig. 3 Schematic of PSLs induced pathway for PGE_2 production in microglia/macrophages. SR-BI dependent PSLs phagocytosis induced ERK1/2 activation, to up-regulate PGE_2 through COX-1/mPGES-2 cascades. The PSLs increased PGE_2 suppresses the phagocytes activation. *SR-BI* class B scavenger receptor type I, *ERK1/2* extracellular signal-regulated kinase ½, *COX* cyclooxygenase, *PG* prostaglandin, *cPGES* cytosolic PGE synthase, *mPGES* microsomal PGE synthase

Conclusion

As all mention above, PSLs can inhibit the maturation and shift the myeloid derived phagocytes from pro- to anti-inflammatory phenotype during immune response. The effects of PSLs on myeloid derived phagocytes are mediated by PGE_2 produced through COX-1/mPGES-2 pathway in an autocrine manner. Because PS is a component of mammalian cell membranes, PSLs can be used as potential pharmacological interventions against the inflammatory and immune diseases without any deleterious side effects.

Acknowledgments Studies conducted in our laboratories were supported by Grants-in-Aid for Scientific Research from the Ministry

of Education, Science and Culture, Japan (No. 20592174 to Zhou Wu and No. 20390472 to Hiroshi Nakanishi).

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