Journal of Neuroimmunology 262 (2013) 121–124

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Short communication

Possible involvement of aiPLA$_2$ in the phosphatidylserine-containing liposomes induced production of PGE$_2$ and PGD$_2$ in microglia

Fumiko Takayama [a], Zhou Wu [a], Hong Mei Ma [a,b], Ryo Okada [a], Yoshinori Hayashi [a], Hiroshi Nakanishi [a,*]

[a] Department of Aging Science and Pharmacology, Faculty of Dental Sciences, Kyushu University, Fukuoka 812-8582, Japan
[b] Department of Prosthodontics, Affiliated Stomatological Hospital, China Medical University, China

ARTICLE INFO

Article history:
Received 3 April 2013
Received in revised form 17 June 2013
Accepted 19 June 2013

Keywords:
Microglia
Phosphatidylserine liposomes
PGE$_2$
PGD$_2$
15d-PGJ$_2$
aiPLA$_2$

ABSTRACT

Liposomes containing phosphatidylserine (PSL) produce PGE$_2$ after being phagocytosed by microglia, but the precise underlying mechanism behind it still remains unclear. Here, we showed that liposomes consisting of phosphatidylserine and lysophosphatidylcholine, a lipolysis product of phosphatidylcholine by PLA$_2$, were phagocytosed by microglia, but failed to induce secretion of PGE$_2$. Furthermore, PSL-induced PGE$_2$ secretion was significantly inhibited by MJ33, an aiPLA$_2$ inhibitor, but not by AACOCF$_3$, a cPLA$_2$ inhibitor. PSL also produced PGD$_2$ and 15d-PGJ$_2$ in microglia. We thus hypothesize that free arachidonic acid is supplied through aiPLA$_2$-mediated lipolysis of phagocytosed phosphatidylcholine, leading to the production of PGH$_2$ and its downstream metabolites.

1. Introduction

After being phagocytosed by myeloid-derived phagocytes, including microglia, macrophages and osteoclasts, liposomes containing phosphatidylserine (PSL) are known to actively suppress an inflammatory response by driving them to produce anti-inflammatory molecules including prostaglandin E$_2$ (PGE$_2$) and transforming growth factor-β1 (TGF-β1) (Matsumoto et al., 2001; Huynh et al., 2002; Zhang et al., 2006; Wu et al., 2010; Wu and Nakanishi, 2011). The activation of extracellular signal-regulated protein kinases 1/2 is necessary for PSL-induction of TGF-β1 (Otsuka et al., 2004, 2007). Conversely, PSL induce PGE$_2$ secretion from microglia without induction of either cyclooxygenase (COX)-2 or microsomal prostaglandin synthase-1, but rather utilize the COX-1 pathway (Zhang at al., 2006). However, the mechanism underlying the production of PGE$_2$ by PSL is still not fully understood. In the present study, we provide the first evidence suggesting that free arachidonic acid is supplied through acidic Ca^{2+}-independent phospholipase A$_2$ (aiPLA$_2$) mediated lipolysis of phagocytosed phosphatidylcholine to the COX-1 signaling pathway, leading to the production of PGH$_2$ and its downstream metabolites, including PGE$_2$, PGD$_2$ and 15-deoxy-$\Delta^{12,14}$-PGJ$_2$ (15d-PGJ$_2$).

2. Materials and methods

2.1. Microglial cell cultures

A c-*myc*-immortalized mouse microglial cell line, MG6 (Transgenic Animal Research Center, National Institute of Agrobiological Science, Tsukuba, Japan) (Takenouchi et al., 2005; Nakamichi et al., 2006), was maintained as previously described (Sun et al., 2012). Primary cultured microglia were prepared from the cerebral cortex of 3-day-old Wistar rats according to previous methods (Sastradipura et al., 1998).

2.2. Preparation of liposomes

The liposomes were composed of either phosphatidylserine combined with phosphatidylcholine (Avanti Polar Lipids, Alabaster, AL, USA) or lysophosphatidylcholine (Sigma Chemical Co., St. Louis, MO, USA) at a molar ratio of 3:7 as described previously (Zhang et al., 2006; Wu et al., 2010). In some experiments, 4-nitrobenz-2-oxa-1,3-diazole (NBD)-labeled phosphatidylserine was used for preparation of PSL.

2.3. Enzyme immunoassay (EIA)

The amounts of PGE$_2$ (GE Healthcare UK Ltd, Amersham, UK) and PGF$_{2\alpha}$ (Cayman Chemical, Ann Arbor, MI, USA) in the culture medium of microglia (5.0×10^5 cells/ml) at 3 h after treatment with PSL (100 μM) or phosphatidylserine/lysophosphatidylcholine liposomes (100 μM) were measured by EIA. The amounts of PGD$_2$ (Cayman

* Corresponding author. Tel.: +81 92 642 6413; fax: +81 92 642 6415.
E-mail address: nakan@dent.kyushu-u.ac.jp (H. Nakanishi).

0165-5728/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jneuroim.2013.06.011

Chemical) and 15d-PGJ_2 (Abnova, Taiwan) in the culture medium of microglia (5.0×10^5 cells/ml) at 24 h after treatment with PSL (100 μM) were measured by EIA.

2.4. Real time RT-PCR

For real-time RT-PCR, the total RNA from primary cultured rat microglia and alveolar macrophages was extracted and purified (Yamada et al., 2006). The thermal cycling was held at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 30 s. After amplifying the PCR products, a melting curve analysis was performed from 55 to 95 °C (0.5 °C/s) to check the synthesized PCR products. The primer sequences were as follows: $aiPLA_2$ sense, 5′-ACATGCTCTACTTTCTGCAGCGG-3′, antisense, 5′-AGAAGCACACGTTTCAGATA-3′; and β-actin sense, 5′-AAGTACCCCATTGAACACGG-3′, antisense, 5′-ATCACAATGCCAGTGGTACG-3′. All real-time RT-PCR experiments were repeated three times and represented as the means of concentration ratio (target gene/β-actin) ± SEM.

2.5. Immunoblot analysis

For the immunoblot analysis, microglia in the culture dishes were treated with PSL (100 μM) for 24 h and mechanically removed after washing with PBS. Proteins were separated on a 15% SDS-polyacrylamide gel and anti-hematopoietic prostaglandin D synthase antibody (H-PGDS; 1:1000; Cayman Chemical) was used for the primary antibody.

2.6. Reagents

Lipopolysaccharide (LPS, from *Escherichia coli* 055:B5), NS-398, indomethacin and bafilomycin A_1 (BFA) were purchased from Sigma. Arachidonyltrifluoromethyl ketone ($AACOCF_3$, Biomol International, Philadelphia, PA, USA), MJ33 (Sigma) and HQL-79 (Cayman Chemical) were also used in this study.

2.7. Statistical analysis

The data are represented as the means ± SEM. The statistical analyses were performed using one-way ANOVA with a post hoc Tukey's test and a value of $P < 0.05$ was considered to indicate statistical significance (GraphPad Software). For immunoblot analyses, a paired Student's *t*-test and a value of $P < 0.05$ was considered to indicate statistical significance.

3. Results and discussion

After being phagocytosed by primary cultured microglia, PSL significantly increased PGE_2 secretion (Fig. 1A, B) as reported previously (Zhang et al., 2006). In contrast, liposomes consisting of

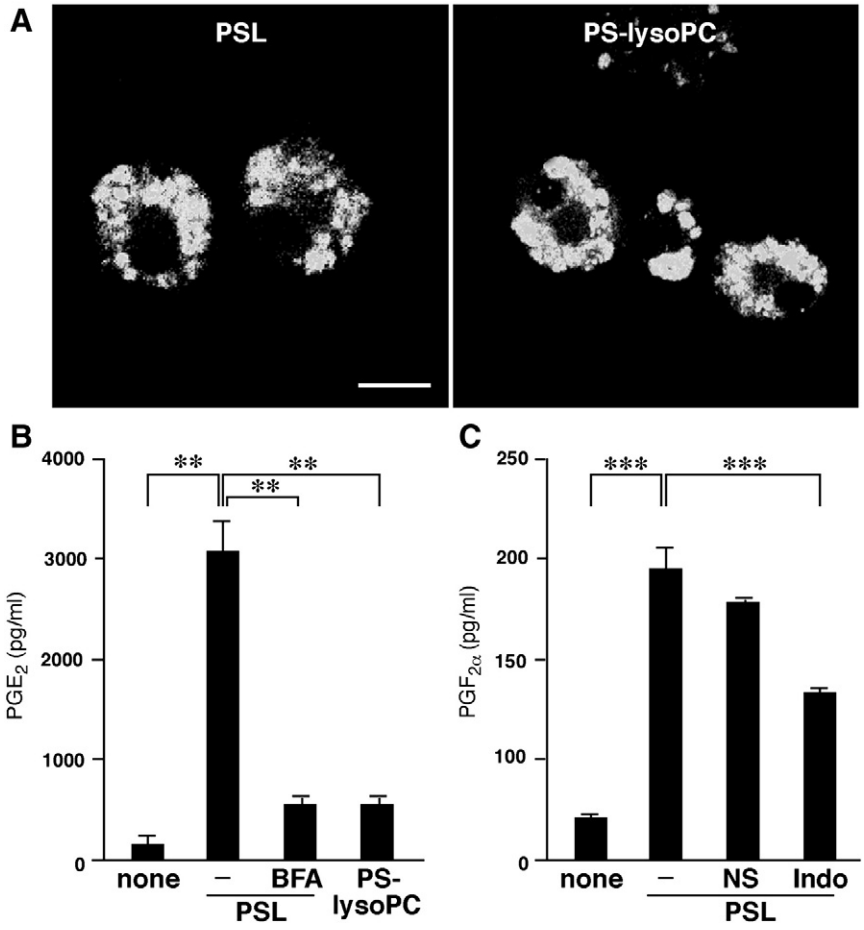

Fig. 1. Effects of PSL and phosphatidylserine/lysophosphatidylcholine (PS-lysoPC) liposomes on the production of PGE_2 in microglia. A. Microglial engulfment of NBD-labeled PSL or PS-lysoPC liposomes. B. Effects of PSL and PS-lysoPC liposomes (100 μM) on the secretion of PGE_2 from microglia. The amounts of PGE_2 in the culture medium were determined at 3 h after treatment with PSL or PS-lysoPC liposomes. The amount of PGE_2 after treatment with PSL was also determined in the presence of BFA (30 μM), a vacuolar H^+-ATPase inhibitor. BFA was applied 1 h before and during treatment with PSL. Each column and bar represents the means ± SEM of three independent experiments. Asterisks indicate significant difference between the values (**$P < 0.01$, one-way ANOVA). C. Effect of PSL on secretion of $PGF_{2\alpha}$ by microglia. The amounts of $PGF_{2\alpha}$ in the culture medium were determined at 3 h after treatment with PSL. The amount of $PGF_{2\alpha}$ after treatment with PSL was also determined in the presence of indomethacin (1 μM) or NS-398 (10 μM). They were applied 1 h before and during treatment with PSL. Each column and bar represents the means ± SEM of three independent experiments. Asterisks indicate significant difference between the values (***$P < 0.001$, one-way ANOVA).