Biochemical and Biophysical Research Communications 374 (2008) 147–151

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Lysophosphatidylserine stimulates chemotactic migration in U87 human glioma cells

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ARTICLE INFO

Article history:
Received 23 June 2008
Available online 9 July 2008

Keywords:
Lysophosphatidylserine
Glioma
Chemotaxis
Receptor

ABSTRACT

Lysophosphatidylserine (LPS) was found to stimulate intracellular calcium increase in U87 human glioma cells. LPS also stimulated chemotactic migration of U87 human glioma cells, which was completely inhibited by pertussis toxin (PTX). Moreover, LPS was also found to stimulate ERK, p38 MAPK, JNK, and Akt activities in U87 cells. We observed that LPS-induced U87 chemotaxis was mediated by PI3K, p38 MAPK, and JNK. LPS-induced chemotactic migration in U87 cells was inhibited by Ki16425, an $LPA_{1/3}$ receptor-selective antagonist, which suggested that the Ki16425-sensitive G-protein coupled receptor (GPCR) played a role in this process. Moreover, U87 cells were found to uniquely express LPA_1 but not LPA_{2-5}. In addition, LPS failed to stimulate the NF-κB-driven luciferase activity in exogenously LPA_1-transfected HepG2 cells. Taken together, we propose that LPS stimulates GPCR, which is in contrast to the well-known LPA receptors, thus resulting in the chemotactic migration in U87 human glioma cells.

The generation of lysophosphatidylserine (LPS) can occur via the activation of serine-phospholipid-selective phospholipase, which is secreted by activated platelets [1]. Previous reports have demonstrated that high concentrations of LPS were found in the ascites of ovarian cancer patients and in the lacrimal fluid after corneal injury [2,3]. In terms of the biological activity of LPS, a previous report has shown that LPS induces a transient increase in intracellular calcium in ovarian and breast cancer cell lines [2]. LPS also has been reported to stimulate IL-2 production in Jurkat T cells, as well as inhibit Jurkat cell proliferation [4]. LPS treatment also enhanced the NGF-induced histamine release in rat mast cells and the NGF-induced differentiation of PC12 cells [5,6]. We previously reported that LPS selectively stimulates human leukemic cells but not human primary leukocytes, thus resulting in intracellular calcium increase [7]. We also demonstrated that LPS induced cell migration in L2071 mouse fibroblasts [8].

Gliomas are well known as highly malignant and resistant to conventional cancer therapy [9]. In addition, chemotactic migration and invasion of tumor cells are closely associated with the high malignancy of gliomas [9]. The chemotactic migration of tumor cells towards a certain microenvironment is a crucial step for the initiation of invasion in certain pathologic events involving cancer cells. Although certain extracellular stimuli such as chemokines and lysophospholipids (i.e., lysophosphatidic acid) have been found to regulate the chemotactic migration of glioma cells [10–12], the extracellular signals that regulate glioma cell migration have not been fully identified. This study addresses the functional role of LPS in U87 human glioma cells. We also characterized the signaling pathways and the role of LPA receptors in the process.

Materials and methods

Cell line and reagents. U87 human glioma cells were cultured in RPMI 1640 medium with 10% FBS, 1% sodium bicarbonate buffer, and 1% HEPES buffer. 1-Acyl-2-hydroxy-*sn*-glycero-3-phospho-L-serine, 1-acyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine, and lysophosphatidic acid were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Fura-2 pentaacetoxymethylester (fura-2/AM) was purchased from Molecular Probes (Eugene, OR). Pertussis toxin (PTX), LY294002, PD98059, SB203580, and SP600125 were purchased from Calbiochem (San Diego, CA). Enhanced chemiluminescence reagents from Amersham Biosciences (Piscataway, NJ), anti-phospho-ERK1/2, anti-phospho-p38 MAPK and anti-phospho-JNK antibodies were purchased from New England Biolabs (Beverly, MA). Ki16425, anti-phospho-Akt antibody, and anti-actin antibody were purchased from Sigma (St. Louis, MO).

Ca^{2+} measurement. Intracellular calcium concentration was determined by Grynkiewicz's method using fura-2/AM [13,14]. Briefly, prepared cells were incubated with 3 μM fura-2/AM at 37 °C for 50 min in fresh serum free RPMI 1640 medium with

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doi:10.1016/j.bbrc.2008.06.117

continuous stirring. Cells (2×10^6) were aliquoted for each assay into Locke's solution (154 mM NaCl, 5.6 mM KCl, 1.2 mM $MgCl_2$, 5 mM HEPES, pH 7.3, 10 mM glucose, 2.2 mM $CaCl_2$, and 0.2 mM EGTA). Fluorescence was measured at 500 nm at excitation wavelengths of 340 nm and 380 nm.

Stimulation of cells with LPS for Western blot analysis. Cultured cells (2×10^6) were stimulated with the indicated concentrations of LPS for the predetermined lengths of time. After stimulation, the cells were washed with serum free RPMI 1640 medium and lysed in lysis buffer (20 mM HEPES, pH 7.2, 10% glycerol, 150 mM NaCl, 1% Triton X-100, 50 mM NaF, 1 mM Na_3VO_4, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride). Detergent insoluble materials were pelleted by centrifugation (12,000*g*, 15 min, at 4 °C), and the soluble supernatant fraction was removed and stored at either −80 °C or used immediately. Protein concentrations in the lysates were determined using Bradford protein assay reagent.

Electrophoresis and immunoblot analysis. Protein samples were prepared for electrophoresis then separated using a 10% SDS–polyacrylamide gel and the buffer system described previously [15]. Following the electrophoresis, the proteins were blotted onto nitrocellulose membrane, which was blocked by incubating with TBST (Tris-buffered saline, 0.05% Tween 20) containing 5% non-fat dried milk. The membranes were then incubated with anti-phospho-ERK antibody, anti-phospho-p38 MAPK antibody, anti-phospho-JNK antibody, anti-phospho-Akt antibody or anti-actin antibody and washed with TBST. Antigen–antibody complexes were visualized after incubating the membrane with 1:5000 diluted goat anti-rabbit IgG antibody coupled to horseradish peroxidase using the enhanced chemiluminescence detection system.

Chemotaxis assay. Chemotaxis assays were performed using multiwell chambers (Neuroprobe Inc., Gaithersburg, MD) as described previously [14,15]. Briefly, polycarbonate filters (10 μm pore size) were precoated with 20 μg/ml of collagen (type II) in HEPES-buffered RPMI 1640 medium. A dry coated filter was placed on a 96-well chamber containing different concentrations of lipids. U87 cells were suspended in RPMI 1640 medium at a concentration of 1×10^6 cells/ml, and 25 μl of the cell suspension were placed onto the upper well of the chamber. After incubation for 4 h at 37 °C, non-migrating cells were removed by scarping, and cells that migrated across the filter were dehydrated, fixed, and stained with hematoxylin (Sigma, St. Louis, MO). The stained cells in five randomly chosen high power fields (HPF, 400×) were then counted for each well. The results are presented as chemotaxis index, which is defined as the number of migrating cells in the presence of test factors over the number of migrating cells in the presence of vehicle.

RT-PCR analysis. mRNA was isolated by using a QIAshredder and an RNeasy kit (Qiagen, Hilden, Germany). mRNA, M-MLV reverse transcriptase, and pd(N)6 primers (Invitrogen Corp., Carlsbad, CA) were used to obtain cDNA. The sequences of the primers used were as follows: LPA_1 receptor: forward, 5′-TCT TCT GGG CCA TTT TCA AC-3′; reverse, 5′-TGC CTR AAG GTG GCG CTC AT-3′. LPA_2 receptor: forward, 5′-CCT ACC TCT TCC TCA TGT TC-3′; reverse, 5′-TAA AGG GTG GAG TCC ATC AG-3′. LPA_3 receptor: forward, 5′-GGA ATT GCC TCT GCA ACA TCT-3′; reverse, 5′-GAG TAG ATG ATG GGG TTC A-3′. LPA_4 receptor: forward, 5′-GGC TTT GTG GTC AAA GGT GT-3′; reverse, 5′-TGC GCT CCC AAG CTA TTA CT-3′. LPA_5 receptor: forward, 5′-CAA AGT GGG ATT GGG AGC TA-3′; reverse, 5′-GCT GAT GAA GCT GTG ACC AA-3′. GAPDH: forward, 5′-GAT GAC ATC AAG AAG GTG GTG AA-3′, reverse, 5′-GTC TTA CTC CTT GGA GGC CAT GT-3′. Amplification was performed over 35 cycles {94 °C/1 min (denaturation), 60 °C/1 min (annealing), and 72 °C/1 min (extension)}. PCR products were electrophoresed on a 2% agarose gel and visualized by ethidium bromide staining.

Luciferase assay. Human LPA_1 cDNAs was a gift from P.G. Suh (POSTECH, Korea). NF-κB reporter construct was purchased from Clonetech (Palo Alto, CA). HepG2 cells were plated onto six-well plates at a density of 5×10^5 cells/well and grown overnight. Cells were transfected with 2 μg of each plasmid construct for 6 h by the Lipofectamine method. After transfection, HepG2 cells were cultured in 10% FBS containing RPMI 1640 medium with LPA (10 μM) or LPS (2 μM) for 20 h. Cells were lysed with lysis buffer (20 mM Tris–HCl, pH 7.8, 1% Triton X-100, 150 mM NaCl, 2 mM DTT). The cell lysate 5 μl was mixed with luciferase activity assay reagent 25 μl and luminescence produced for 5 s was measured using luminoskan (labsystems).

Statistics. The results are expressed as means ± SE of the number of determinations indicated. Statistical significance of differences was determined by Student *t*-test. Significance was accepted when $P < 0.05$.

Results

LPS stimulates calcium mobilization in U87 human glioma cells

We tested the effects of LPS on intracellular calcium concentration ($[Ca^{2+}]_i$) in U87 human glioma cells. The stimulation of U87 cells at various concentrations (0, 0.1, 0.5, 1, 2, and 5 μM) of LPS caused $[Ca^{2+}]_i$ elevation in U87 cells (Fig. 1A). LPA was also found to stimulate $[Ca^{2+}]_i$ increase in a concentration-dependent manner in these cells (Fig. 1A). Increase of $[Ca^{2+}]_i$ was apparent at 100 nM of LPS and maximal activity was observed at 1 μM (Fig. 1A). We also investigated the role of PTX-sensitive G proteins on LPS-induced $[Ca^{2+}]_i$ elevation. To achieve this, we preincubated U87 cells with 100 ng/ml of PTX, prior to stimulation, with 2 μM LPS. The preincubation of PTX prior to LPS stimulation dramatically inhibited the $[Ca^{2+}]_i$ elevation by LPS (Fig. 1B), thus suggesting that LPS induces $[Ca^{2+}]_i$ elevation in a PTX-sensitive manner.

LPS induces U87 chemotaxis

The chemotactic migration of gliomas is an important process for their pathogenesis [9]. Consequently, we examined the effect of LPS on U87 human glioma migration. As shown in Fig. 2A, the stim-

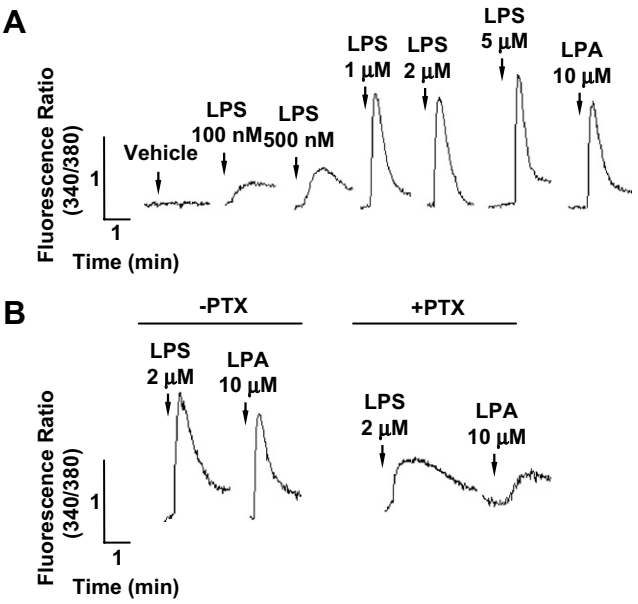

Fig. 1. Effect of LPS on $[Ca^{2+}]_i$ increase in U87 human glioma cells. U87 cells were stimulated with various concentrations of LPS (0, 0.1, 0.5, 1, 2, and 5 μM) or 10 μM of LPA (A). Calcium concentration was determined on preincubated U87 cells in the absence or presence of 100 ng/ml of PTX for 24 h with LPS (2 μM) or LPA (10 μM) (B). The changes in the absorbance at 340 nm and 380 nm were measured. The results are representative of three independent experiments (A,B).

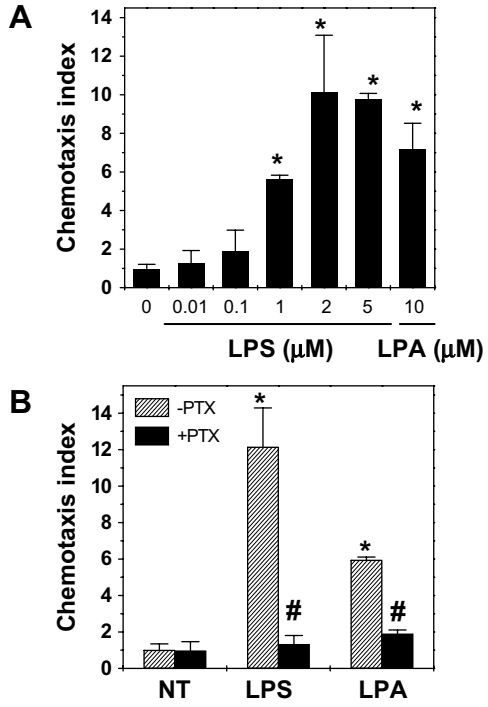

Fig. 2. LPS induces U87 chemotaxis via PTX-sensitive G-protein. Cultured U87 cells (1×10^6 cells/ml in serum free RPMI) were added to the upper well of a 96-well chemotaxis chamber and assessed for migration across a polycarbonate membrane (10 μm pore size) in the presence of various concentrations (0, 0.01, 0.1, 0.5, 1, 2, and 5 μM) of LPS for 4 h at 37 °C (A). U87 cells preincubated in the absence or presence of PTX (100 ng/m) for 24 h were subjected to chemotaxis assays in the presence of LPS (2 μM) or LPA (10 μM) (B). The number of cells that migrated was determined by counting 5 high power fields (400×). The results are shown as the average chemotaxis index. The data are presented as means ± SE of three independent experiments performed in duplicate (A,B). *Significantly different from the control (NT) ($P < 0.05$). #Significantly different from the control (-PTX) ($P < 0.05$).

ulation of U87 cells at various concentrations of LPS induced the chemotactic migration of U87 cells. The concentration-responsive curve of LPS-induced U87 cell migration indicated that the maximal activity occurred at 2–5 μM (Fig. 2A). To distinguish between LPS-induced chemotaxis and chemokinesis, we performed migration assays in the absence or presence of LPS in the upper wells of chemotaxis chambers, as described previously [8]. The addition of LPS (10 μM) in the upper chamber was found to reduce the LPS-induced migrations of U87 cells to the lower, which demonstrated that LPS induced U87 cell chemotaxis (data not shown).

Since the LPS-induced calcium increase was inhibited by PTX in U87 cells, we examined the effect of PTX on LPS-induced U87 human glioma chemotaxis. When U87 cells were preincubated with 100 ng/ml of PTX prior to the chemotaxis assay, the number of cells migrating toward LPS was reduced by greater than 99% when compared to cells without PTX treatment (Fig. 2B), thus strongly suggesting the involvement of PTX-sensitive G proteins in the process.

LPS stimulates ERK, p38 MAPK, JNK, and Akt activity in U87 cells

Some protein kinases such as MAPK have been reported to mediate extracellular signals, thus resulting in several cellular responses including cell trafficking [16–18]. Here, we used a Western blot analysis with anti-phospho-specific antibodies against each enzyme to examine whether LPS stimulates MAPKs. When U87 cells were stimulated with 2 μM of LPS at different times (0, 2, 5, 10, and 30 min), the phosphorylation level of ERK was transiently increased, with the maximal activity occurring at 10 min after stimulation (Fig. 3A), and the return to baseline occurring at 30 min after stimulation (Fig. 3A). Other important MAPKs, p38 MAPK, and JNK were also transiently activated by LPS stimulation in a time-dependent manner (Fig. 3A).

Akt has been reported to play an important role in the regulation of various cellular responses including cell migration and survival [19]. Here, we used the Western blot analysis with anti-phospho-specific antibodies against Akt to determine whether LPS stimulates Akt. When U87 cells were stimulated with 2 μM of LPS at different times, the phosphorylation of Akt was increased after 5 min of stimulation, which was sustained until 30 min after stimulation (Fig. 3A).

Since we observed that LPS treatment led to a rapid increase in the phosphorylation of three different MAPKs and Akt in U87 cells (Fig. 3A), we subsequently investigated whether the MAPK and PI3K pathways are required for LPS-induced U87 cell chemotaxis. The preincubation of cells with LY294002 (50 μM), a well-known PI3K inhibitor, for 15 min at 37 °C prior to stimulation with LPS was

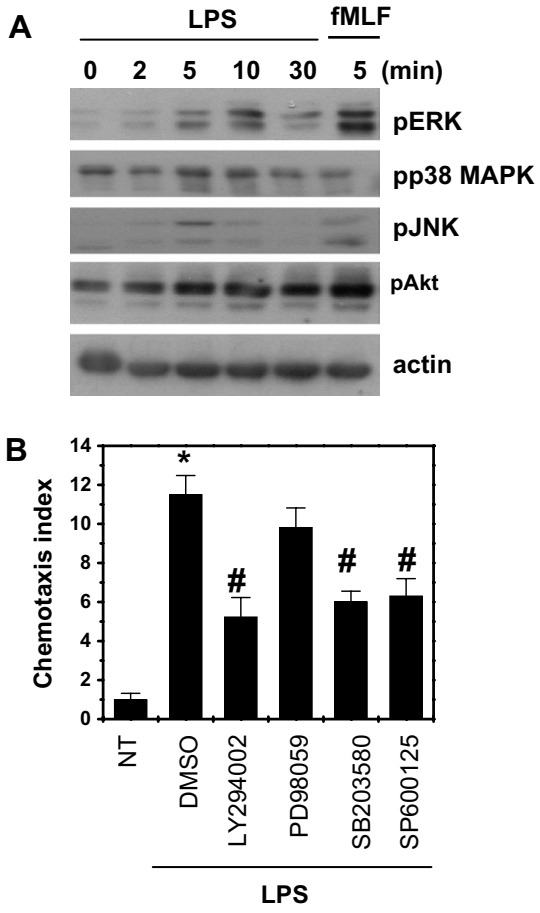

Fig. 3. LPS-induced U87 chemotaxis is mediated by p38 MAPK, JNK, and PI3K. U87 cells were stimulated with 2 μM of LPS at various times (0, 2, 5, 10, and 30 min) or 1 μM of fMLF for 5 min (A,B). Each sample (30 μg of protein) was subjected to 10% SDS-PAGE, and phospho-ERK (pERK), phospho-p38 MAPK (pp38 MAPK), phospho-JNK (pJNK), or phospho-Akt (pAkt) levels were determined by immunoblotting using anti-phospho-ERK, anti-phospho-p38 MAPK, anti-phospho-JNK, or anti-phospho-Akt antibodies (A). The results shown are representative of at least three independent experiments (A). U87 cells were treated with vehicle (DMSO), LY294002 (50 μM), PD098059 (50 μM), SB203580 (20 μM), or SP600125 (10 μM) for 15 min (60 min for PD98059), and then subjected to chemotaxis assays in the presence of 2 μM LPS for 4 h (B). Migrated cell counts were determined by enumerating cells in 5 high power fields (400×). The results are shown as the average chemotaxis index. The data are presented as means ± SE of six independent experiments performed in duplicate (C). *Significantly different from the control (NT) ($P < 0.05$). #Significantly different from the control (DMSO treated) ($P < 0.05$).

found to affect cellular chemotaxis (Fig. 3B), thus indicating that LPS activates the PI3K pathway and that this signaling is required for the LPS-induced chemotaxis of U87 human glioma cells.

We also examined the roles of ERK, p38 MAPK, or JNK on LPS-induced U87 chemotaxis. Following the preincubation of U87 cells with PD98059 (50 μM), SB203580 (20 μM), or SP600125 (10 μM) prior to the chemotaxis assay, the LPS-induced U87 chemotaxis was found to be significantly blunted by SB203580, SP600125, but not by PD98059 (Fig. 3B). This implies that p38 MAPK and JNK-mediated signaling are involved in LPS-induced U87 chemotaxis.

LPS-induced chemotactic migration is Ki16425-sensitive but independent of LPA_1

Following the finding indicating that LPS stimulates chemotactic migration in a PTX-sensitive manner (Fig. 2), we examined the effect of an LPA receptor antagonist (Ki16425) on LPS-induced chemotaxis in U87 cells. The preincubation of U87 cells with 5 μM of Ki16425 (an LPA_1 and LPA_3-selective antagonist) was found to inhibit LPA-induced U87 cell chemotaxis (Fig. 4A). Moreover, LPS-stimulated chemotaxis was also completely blocked by Ki16425 (Fig. 4A). The results indicate that LPS stimulates chemotaxis in U87 cells through a Ki16425-sensitive GPCR.

Next, we investigated which types of LPA receptors are expressed in U87 cells via RT-PCR analysis. As shown in Fig. 4B, U87 cells expressed one of the LPA receptors; LPA_1 but not LPA_{2-5}. To test whether LPS stimulates U87 cells via LPA_1, we utilized the nuclear factor kappaB (NF-κB)-driven luciferase activity. At first, we observed that the treatment with LPA or LPS induced the NF-κB-driven luciferase activity in NF-κB-luciferase transfected U87 cells (data not shown). Next, we investigated the effect of LPA or LPS on NF-κB activity in LPA_1-transfected HepG2 cells. As shown in Fig. 4C, treatment with LPA elicited NF-κB-driven luciferase activity in LPA_1-transfected HepG2 cells. However, LPS failed to stimulate NF-κB-driven luciferase activity in the cells (Fig. 4C). These findings strongly indicate that LPS is not an agonist for the known LPA receptor, LPA_1.

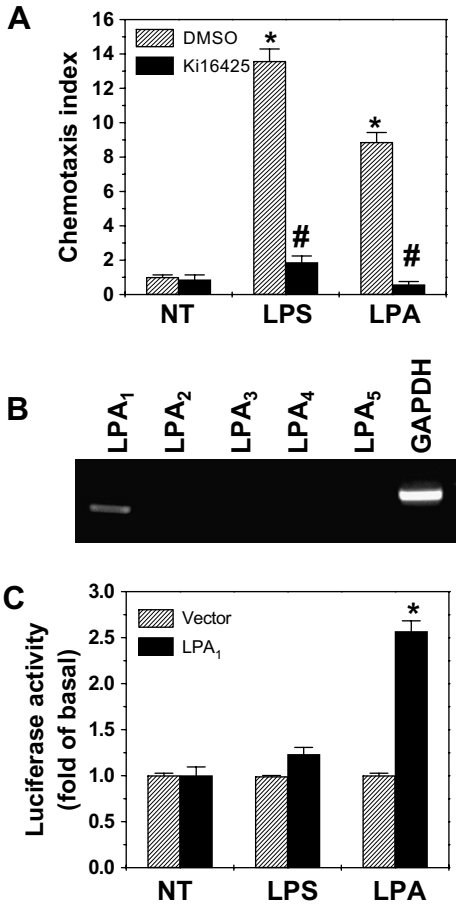

Fig. 4. LPS-induced U87 chemotaxis is mediated Ki16425-sensitive receptor. U87 cells were treated with vehicle (DMSO) or Ki16425 (10 μM) for 15 min, and then subjected to chemotaxis assays in the presence of 2 μM LPS or 10 μM LPA for 4 h. Migrated cell counts were determined by enumerating cells in 5 high power fields (400×). The results are shown as the average chemotaxis index (A). mRNA was isolated from U87 cells. Semiquantitative RT-PCR was performed to determine LPA_{1-5} mRNA expressions. The data shown are representative of three independent experiments (B). LPA_1, cDNA, and NF-κB-luciferase cDNA were co-transfected into HepG2 cells. Cells were stimulated with LPA (10 μM) or LPS (2 μM) for 20 h (C). Luciferase activities were measured in cell lysates using luciferase reporter gene assays. The data shown represent means ± SE of three independent experiments performed in duplicate (A,C). *Significantly different from the control (NT) ($P < 0.05$). #Significantly different from the control (DMSO treated) ($P < 0.05$).

Discussion

Glioma migration is associated with the metastasis of the cancer cells. As a result, the modulation of chemotactic migration is an important aspect of cancer biology. Several groups have reported that various extracellular stimuli are involved in the regulation of glioma chemotactic migration. However, the role of LPS in the chemotaxis of gliomas has not been studied. Here, we report that LPS stimulates chemotactic migration in U87 human glioma cells. From these results, we propose that LPS can modulate the pathogenesis of glioma by stimulating chemotactic migration.

In addition to the biological role of LPS in human glioma cells, the sensitivity of LPS-induced chemotaxis to Ki16425 was also determined. In a previous report, we showed that LPS-stimulated chemotaxis in fibroblasts was not inhibited by VPC32183 (an LPA_1- and LPA_3-selective antagonist), although LPA-induced chemotaxis were inhibited by VPC32183 [8]. However, we found a contradictory result indicating that LPS-induced chemotactic migration was completely inhibited by Ki16425 (an LPA_1- and LPA_3-selective antagonist) in U87 cells (Fig. 4), thereby implying that Ki16425-sensitive GPCRs are involved in the LPS action of glioma cells. We also found that LPS failed to stimulate NF-κB-driven luciferase activity in HepG2 cells expressing LPA_1 exogenously, thus suggesting that LPS has its own receptor which is distinct from LPA's (Fig. 4C). These results suggest that LPS may not act on Ki16425-sensitive LPA receptors such as LPA_1 or LPA_3. Moreover, LPS seems to indicate the existence of two GPCR subtypes; one being the VPC32183-insensitive GPCR which is coupled to PTX-sensitive G proteins and expressed in L2071 cells. The other is the Ki16425-sensitive GPCR, which is coupled to PTX-sensitive G proteins and expressed in U87 cells. Although the sensitivity of LPA_4 and LPA_5 to Ki16425 has not yet been fully studied, since we observed that U87 cells do not express LPA_4 and LPA_5 in mRNA level, we are able to exclude the possibility that the Ki16425-sensitive LPS receptor in U87 cells is a LPA receptor, including LPA_4 and LPA_5. GPR34 has also been recognized as a specific cell surface receptor for LPS in mast cells [20]. In this study, we investigated whether U87 cells express GPR34 by RT-PCR analysis, and found that U87 cells do not express GPR34 (data not shown). As a result, we can rule out the possibility that LPS stimulates U87 chemotaxis via GPR34.

In conclusion, we demonstrated that LPS stimulates chemotactic migration via the PTX-sensitive and Ki16425-sensitive cell surface receptors. Our findings are expected shed light on the biological roles and findings of pertaining to the cell surface receptors of LPS in future studies.

Acknowledgments

This work was supported by the Korea Science and Engineering Foundation through the Medical Science and Engineering Research Center for Cancer Molecular Therapy at Dong-A University.

References

[1] T. Sato, J. Aoki, Y. Nagai, N. Dohmae, K. Takio, T. Doi, H. Arai, K. Inoue, Serine phospholipid-specific phospholipase A that is secreted from activated platelets. A new member of the lipase family, J. Biol. Chem. 272 (1997) 2192–2198.
[2] Y. Xu, X.J. Fang, G. Casey, G.B. Mills, Lysophospholipids activate ovarian and breast cancer cells, Biochem. J. 309 (1995) 933–940.
[3] K. Liliom, Z. Guan, J.L. Tseng, D.M. Desiderio, G. Tigyi, M.A. Watsky, Growth factor-like phospholipids generated after corneal injury, Am. J. Physiol. 274 (1998) C1065–C1074.
[4] Y. Xu, G. Casey, G.B. Mills, Effect of lysophospholipids on signaling in the human Jurkat T cell line, J. Cell. Physiol. 163 (1995) 441–450.
[5] K. Kawamoto, J. Aoki, A. Tanaka, A. Itakura, H. Hosono, H. Arai, Y. Kiso, H. Matsuda, Nerve growth factor activates mast cells through the collaborative interaction with lysophosphatidylserine expressed on the membrane surface of activated platelets, J. Immunol. 168 (2002) 6412–6419.
[6] S. Lourenssen, M.G. Blennerhassett, Lysophosphatidylserine potentiates nerve growth factor-induced differentiation of PC12 cells, Neurosci. Lett. 248 (1998) 77–80.
[7] K.S. Park, H.Y. Lee, M.K. Kim, E.H. Shin, Y.S. Bae, Lysophosphatidylserine stimulates leukemic cells but not normal leukocytes, Biochem. Biophys. Res. Commun. 333 (2005) 353–358.
[8] K.S. Park, H.Y. Lee, M.K. Kim, E.H. Shin, S.H. Jo, S.D. Kim, D.S. Im, Y.S. Bae, Lysophosphatidylserine stimulates L2071 mouse fibroblast chemotactic migration via a process involving pertussis toxin-sensitive trimeric G-proteins, Mol. Pharmacol. 69 (2006) 1066–1073.
[9] F.B. Furnari, T. Fenton, R.M. Bachoo, A. Mukasa, J.M. Stommel, A. Stegh, W.C. Hahn, K.L. Ligon, D.N. Louis, C. Brennan, L. Chin, R.A. DePinho, W.K. Cavenee, Malignant astrocytic glioma: genetics, biology, and paths to treatment, Genes Dev. 21 (2007) 2683–2710.
[10] S.A. Rempel, S. Dudas, S. Ge, J.A. Gutiérrez, Identification and localization of the cytokine SDF1 and its receptor, CXC chemokine receptor 4, to regions of necrosis and angiogenesis in human glioblastoma, Clin. Cancer Res. 6 (2000) 102–111.
[11] A. Bajetto, F. Barbieri, A. Dorcaratto, S. Barbero, A. Daga, C. Porcile, J.L. Ravetti, G. Zona, R. Spaziante, G. Corte, G. Schettini, T. Florio, Expression of CXC chemokine receptors 1–5 and their ligands in human glioma tissues: role of CXCR4 and SDF1 in glioma cell proliferation and migration, Neurochem. Int. 49 (2006) 423–432.
[12] T.J. Manning Jr., J.C. Parker, H. Sontheimer, Role of lysophosphatidic acid and rho in glioma cell motility, Cell Motil. Cytoskeleton 45 (2000) 185–199.
[13] G. Grynkiewicz, M. Poenie, R.Y. Tsien, A new generation of Ca2+ indicators with greatly improved fluorescence properties, J. Biol. Chem. 260 (1985) 3440–3450.
[14] Y.S. Bae, H. Bae, Y. Kim, T.G. Lee, P.G. Suh, S.H. Ryu, Identification of novel chemoattractant peptides for human leukocytes, Blood 97 (2001) 2854–2862.
[15] Y.S. Bae, H.J. Yi, H.Y. Lee, E.J. Jo, J.I. Kim, T.G. Lee, R.D. Ye, J.Y. Kwak, S.H. Ryu, Differential activation of formyl peptide receptor-like 1 by peptide ligands, J. Immunol. 171 (2003) 6807–6813.
[16] G.T. Kampen, S. Stafford, T. Adachi, T. Jinquan, S. Quan, J.A. Grant, P.S. Skov, L.K. Poulsen, R. Alam, Eotaxin induces degranulation and chemotaxis of eosinophils through the activation of ERK2 and p38 mitogen-activated protein kinases, Blood 95 (2000) 1911–1917.
[17] A. Bonacchi, P. Romagnani, R.G. Romanelli, E. Efsen, F. Annunziato, L. Lasagni, M. Francalanci, M. Serio, G. Laffi, M. Pinzani, P. Gentilini, F. Marra, Signal transduction by the chemokine receptor CXCR3: activation of Ras/ERK, Src, and phosphatidylinositol 3-kinase/Akt controls cell migration and proliferation in human vascular pericytes, J. Biol. Chem. 276 (2001) 9945–9954.
[18] M.S. Kim, G. Kewalramani, P. Puthanveetil, V. Lee, U. Kumar, D. An, A. Abrahani, B. Rodrigues, Acute diabetes moderates trafficking of cardiac lipoprotein lipase through p38 mitogen-activated protein kinase-dependent actin cytoskeleton organization, Diabetes 57 (2008) 64–76.
[19] M. Morales-Ruiz, M.J. Lee, S. Zollner, J.P. Gratton, R. Scotland, I. Shiojima, K. Walsh, T. Hla, W.C. Sessa, Sphingosine 1-phosphate activates Akt, nitric oxide production, and chemotaxis through a Gi protein/phosphoinositide 3-kinase pathway in endothelial cells, J. Biol. Chem. 276 (2001) 19672–19677.
[20] T. Sugo, H. Tachimoto, T. Chikatsu, Y. Murakami, Y. Kikukawa, S. Sato, K. Kikuchi, T. Nagi, M. Harada, K. Ogi, M. Ebisawa, M. Mori, Identification of a lysophosphatidylserine receptor on mast cells, Biochem. Biophys. Res. Commun. 341 (2006) 1078–1087.