Gangliosides Activate Cultured Rat Brain Microglia*  

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Microglia, brain resident macrophages, are activated in brain injuries and several neurodegenerative diseases. However, microglial activators that are produced in the brain are not yet defined. In this study, we showed that gangliosides, sialic acid-containing glycosphingolipids, could be a microglial activator. Gangliosides induced production of nitric oxide (NO) and tumor necrosis factor-α (TNF-α) and expression of cyclooxygenase-2 (COX-2). The effect of gangliosides on NO release increased dose-dependently in the range of 10–100 μg/ml; however, the effect decreased at concentrations higher than 200 μg/ml. Specific types of gangliosides showed differential effects on microglial activation. Similar to gangliosides, GT1b induced production of NO and TNF-α and expression of COX-2. However, GM1 and GD1a induced expression of COX-2 but had little effect on NO and TNF-α release. The effect of gangliosides and GT1b on NO release was reduced in the presence of neuraminidase, which removes sialic acid residues from gangliosides and GT1b. Gangliosides activated extracellular signal-regulated kinase significantly but activated c-jun N-terminal kinase/stress-activated protein kinase and p38 relatively weakly. The inhibition of extracellular signal-regulated kinase by PD98059 reduced NO release from both gangliosides- and GT1b-treated microglia whereas inhibition of p38 by SB203580 increased it rather slightly. Gangliosides activated NF-κB, and N-acetyl cysteine, an inhibitor of NF-κB, reduced NO release. These results suggest that gangliosides could be a microglial activator that functions via activation of mitogen-activated protein kinase and NF-κB.

Microglia known as major inflammatory cells of the brain are activated in brain injuries and diseases such as trauma, ischemia, Alzheimer’s disease, and multiple sclerosis (1–3). Activated microglia induced the expression of the genes encoding iNOS, 1 TNF-α, and COX-2, which are responsible for the production of inflammatory mediators, nitric oxide (NO), TNF-α, and prostaglandins, respectively (4–6). These inflammatory mediators potentiated neuronal injury (7, 8). However, it has not been clarified by what microglia are activated in diverse pathological conditions of the brain. Although lipopolysaccharide (LPS), interferon-γ, and beta-amyloid (Aβ) have been known as microglial activators, LPS is a cell-wall component of Gram-negative bacteria, and interferon-γ is produced by T lymphocytes, which are absent in the brain (9, 10). Although Aβ is produced in the brain, it is confined to Alzheimer’s disease. Because microglial activation accompanies brain damage, a component(s) released from injured cells could be involved in microglial activation.

Gangliosides are glycosphingolipid-containing sialic acid residues. Gangliosides exist in mammalian cell membrane and are particularly rich in neuronal cell membrane (11). GM1, GD1a, GD1b, GT1b, and GQ1b are major types of gangliosides found in the brain (12, 13). They are different in the number and sites of sialic acid residues attaching to the carbohydrates. Gangliosides have been reported to participate in proliferation and differentiation of various types of cells. Gangliosides reduced proliferation of Schwann cells (14) and induced differentiation of keratinocytes (15). Gangliosides regulated the function of several inflammatory cells. Gangliosides increased phagocytic activity of human polymorphonuclear leukocytes and microglia (16, 17). GM3 induced iNOS expression in murine peritoneal macrophages (18), and GM1 enhanced production of interleukin-1β from reactive astrocytes (19).

MAPK is at least one of the common intracellular signaling molecules involved in microglial activation. LPS and Aβ activated three subtypes of MAPKs, ERK, p38, and JNK/SAPK. Furthermore, the inhibition of ERK and p38 significantly reduced LPS-induced NO release and Aβ-induced TNF-α release (20). Gangliosides have also been reported to activate MAPKs. GM1 activated ERK2 in human glial cells (21), and GM3-induced CD4 down-regulation was blocked by inhibition of ERK (22). NF-κB could be another common intracellular signaling molecule activated by interferon-γ, Aβ, and LPS (23). The NF-κB-binding sites are found in the promoter regions of iNOS, TNF-α, and COX-2 (24–26), and the expression of these three genes was regulated by the activation of NF-κB (27–29). However, gangliosides have been reported to have the opposite effect on the activation of NF-κB. In T lymphocytes and monocytes, gangliosides prevented the activation of NF-κB (30, 31). However, GD3-mediated activation of c-rel, a subtype of NF-κB (32), was also reported in human blood T lymphocytes (33). In this study, we investigated whether gangliosides could activate microglia and whether gangliosides induced microglial activation via activation of MAPKs and NF-κB as did LPS and Aβ (20, 34).

EXPERIMENTAL PROCEDURES

Preparation of Microglia—Microglia were cultured from the cerebral cortices of 1–3-day old Sprague-Dawley rats as previously described (4, 20). Briefly, the cortices were triturated into single cells in minimal essential medium (Life Technologies, Inc.) containing 10% fetal bovine serum (HyClone, Logan, Utah) and plated into 75 cm² T-flasks (0.5
of each band was analyzed by NIH image 1.61. The relative intensities of ERK (Santa Cruz Biotechnology), JNK1 (Santa Cruz Biotechnology), respectively. The intensity of nitrite released from each treatment was measured by nitrite analysis as described elsewhere (36).

Determination of NO Release—Microglia plated in 24-well plates were treated with 1–300 µg/ml gangliosides (mixture), GM1, GD1a, or GT1b (Matreya, Pleasant Gap, PA) for various time periods. The amount of nitrite converted from NO was determined by using the culture media (50 µl) with an equal volume of Griess reagent (0.1% naphthylethylene diamine, 1% sulfanilamide, 2.5% H3PO4). The optical densities at 540 nm were measured (35). To examine the involvement of MAPKs and NF-κB in NO release, PD98059 (inhibitor of ERK kinase, Calbiochem), SB203580 (inhibitor of p38, Calbiochem), or α-acetylcystein (inhibitor of NF-κB, Sigma) was co-treated with gangliosides or GT1b. To test whether sialic acid residues are important for the action of gangliosides, gangliosides and GT1b were preincubated with 550 milliunits/ml neuraminidase to remove sialic acid residues at 37°C for 2 h.

Determination of TNF-α Release—Microglia plated in 24-well plates were treated with gangliosides (50 µg/ml), GT1b (20 µg/ml), GM1 (50 µg/ml), or GD1a (50 µg/ml). TNF-α released into 50 µl of culture media was measured using enzyme-linked immunosor bent assay kits (BIOSOURCE, Camarillo, CA) following the manufacturer’s instructions.

Determination of iNOS and COX-2 —The expression of iNOS and COX-2 was determined by immunoblot analysis. Microglia (1 × 106 cells) were treated with gangliosides (50 µg/ml), GT1b (10–20 µg/ml), GM1 (50 µg/ml), or GD1a (50 µg/ml) for 12–48 h. The cells were then washed with ice-cold phosphate-buffered saline and lysed with modified radioimmune precipitation buffer lysis buffer (150 mM NaCl, 10 mM Na2HPO4, pH 7.2, 0.5% sodium deoxycholate, 1% Nonidet P-40) containing protease inhibitors (2 mM phenylmethylsulfonyl fluoride, 100 µg/ml leupeptin, 2 mM EDTA, and 10 µg/ml pepstatin). Proteins were separated on a 7.5% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. The membrane was incubated with iNOS antibodies (1:2000 dilution, UBI, Lake Placid, NY) or COX-2 antibodies (1:1000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h. The membrane was washed three times with Tris-buffered saline including 0.05% Tween 20 and incubated with peroxidase-conjugated secondary antibodies (Vector Lab., Burlingame, CA). iNOS and COX-2 were visualized using an enhanced chemiluminescence system.

Measurement of MAPK Activation—Microglia (1 × 106 cells) were incubated in serum-free media overnight and then treated with 50 µg/ml gangliosides or 100 ng/ml LPS (from Salmonella enteritidis, Sigma). Cells were washed three times with ice-cold phosphate-buffered saline, lysed with 2× SDS-polyacrylamide gel electrophoresis sample buffer and then applied to a 10% SDS-polyacrylamide gel. Proteins were transferred onto a polyvinylidene difluoride membrane, and activation of MAPKs was examined by immunoblot analysis using antibodies specific for phosphorylated forms of ERK, p38, and JNK (New England Biolabs, UK). Some membranes were stripped with 0.2 N NaOH for 20 min and used for immunoblot analysis using antibodies against ERK (UBI, Lake Placid, NY), p38 (Santa Cruz Biotechnology, Santa Cruz, CA), and JNK1 (Santa Cruz Biotechnology), respectively. The intensity of each band was analyzed by NIH image 1.61. The relative intensities of phosphorylated ERK, p38, and JNK were normalized and compared to the intensities of unphosphorylated counterbands.

Measurement of NF-κB Activation by Electrophoresis Mobility Shift Assay and Super-Shift Assay—Electrophoresis mobility shift assay was carried out as previously described (36). Microglia (5 × 106 cells) were harvested and suspended in 900 µl of a hypotonic solution (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride) including 0.5% Nonidet P-40 on ice for 5 min. Cells were centrifuged at 500 × g for 10 min at 4 °C, and the pellet (nuclear fraction) was saved. The nuclear fraction was resuspended in a buffer containing 20 mM HEPES, pH 7.9, 20% glycerol, 0.4 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride, incubated on ice for 60 min with occasional gentle shaking, and centrifuged at 12,000 × g for 15 min. The crude nuclear proteins in the supernatant were collected and stored at −70°C for electrophoresis mobility shift assay experiments. Two synthetic oligonucleotides (Genosys, The Woodlands, TX) containing the NF-κB binding sequence of the murine immunoglobulin light chain gene (5-GGGAGTTGAGGGGACTTTCCGAGG-3) and its complementary sequence were end-labeled using Klenow fragment and [α-32P]dCTP. The labeled DNA probe (approximately 0.2 ng) was incubated with 0.5 µg of nuclear proteins in a reaction mixture containing 8.5 mM EDTA, 8.5 mM EGTA, 8% glycerol, 0.1 mM ZnSO4, 50 µg/ml poly(dI-dC), 1 mM dithiothreitol, 0.3 µg/ml bovine serum albumin, and 6 mM MgCl2 for 30 min. The reaction mixture was applied to an 8% polyacrylamide gel. After electrophoresis, the gel was dried and an autoradiogram was obtained. To confirm specific binding of labeled oligonucleotides to the nuclear protein, the excess amount of unlabeled oligonucleotide (5 ng) was added to the reaction mixture, and disappearance of the shifted band was examined. For super-shift assay, the nuclear extract was pre-incubated with 1 µg of anti-p50 or anti-p65 antibodies (Santa Cruz Biotechnology) for 30 min. The reaction mixture was subjected to electrophoresis on a 6% polyacrylamide gel.

RESULTS

Gangliosides Induced NO Release—The effect of gangliosides on NO release was dose-dependent up to 100 µM/ml. (mean ± S.E. of indicated number of samples in figures), 13.4 ± 3.4, 27.0 ± 2.1, and 29.4 ± 1.0 µM NO were detected from cells (5 × 104) treated with 1, 10, 50, and 100 µg/ml gangliosides, respectively, whereas 2.3 ± 0.8 µM was released from untreated cells (Fig. 1A). The release of NO was not because of toxicity of gangliosides on these cells, which was confirmed by morphology of cells under a light microscope. iNOS expression was detected within 12 h after gangliosides treatment and was maintained until 48 h (Fig. 1A, inset). Thus, the effect of gangliosides on NO release was because of the expression of iNOS. NO release from cells treated with 50 µg/ml gangliosides was continuously increased during the experimental period (Fig. 1B). At 12, 24, 48, and 72 h, 5.5 ± 0.6, 9.9 ± 0.3, 18.1 ± 1.1, and 31.0 ± 0.2 µM NO were detected. In contrast, 1.8 ± 0.9, 3.2 ± 0.3, 3.3 ± 0.8, and 7.7 ± 0.3 µM NO were detected from untreated cells at the same time point.

Gangliosides induced TNF-α release. In enzyme-linked immunosor bent assay, TNF-α was detected within four h after microglia (5 × 104) were treated with 50 µg/ml gangliosides. At 4, 10, 24, and 48 h, 975 ± 59, 797 ± 24, 432 ± 22, and 404 ± 18
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Fig. 2. Gangliosides induced TNF-α release. Microglia were treated with 50 μg/ml gangliosides for 4, 10, 24, and 48 h. The amount of TNF-α released into the medium was measured by enzyme-linked immunosorbent assay. Values were mean ± S.E. of three samples.

Fig. 3. Gangliosides-treated microglia expressed COX-2. Microglia were treated with 50 μg/ml gangliosides for 12, 24, and 48 h, and the expression of COX-2 was detected with COX-2 antibodies.

pg/ml TNF-α was detected. However, untreated microglia barely produced TNF-α; 59 ± 5, 105 ± 23, 51 ± 7, and 65 ± 4 pg/ml were detected at the same time point (Fig. 2). The decrease in TNF-α at later time points could be because of degradation of TNF-α in the media.

Gangliosides also induced the expression of COX-2. The increased expression of COX-2 could represent the production of prostaglandins because COX-2 catalyzes the synthesis of prostaglandins (37). COX-2 expression was detected 12 h after 50 μg/ml gangliosides were treated, and expression slightly decreased but was maintained up to 48 h (Fig. 3). These results showed that gangliosides could induce all three inflammatory mediators tested. Thus, gangliosides could be considered as a microglial activator.

Effects of Specific Types of Gangliosides on Microglial Activation—Gangliosides are composed of various types of gangliosides. Thus, we tested the effect of three major types of gangliosides in the brain, GM1, GD1a, and GT1b, on microglial activation. Both GM1 and GD1a had little effect on NO release. Microglia treated with 1.5, 15, 75, and 150 μg/ml GM1 released 2.1 ± 0.5, 6.5 ± 1.3, 4.3 ± 0.8, and 4.9 ± 0.9 μM, respectively. The cells treated with 2, 20, and 100 μg/ml GD1a also released only 4.5 ± 1.1, 3.4 ± 1.4, and 0.0 ± 1.1 μM, respectively (Fig. 4, A and B). However, cells treated with 2 and 10 μg/ml GT1b released 7.0 ± 0.9 and 23.9 ± 3.0 μM of NO, respectively. GT1b showed a similar dose-dependent effect on NO release as did gangliosides. NO release was maximum at 10 μg/ml GT1b but rather decreased at higher concentrations; at 20, 100, and 200 μg/ml GT1b, 16.5 ± 1.9, 4.0 ± 2.1, and −1.1 ± 0.5 μM NO were detected (Fig. 4C). GT1b also induced the expression of iNOS (Fig. 4C, inset). The reduction of NO release at higher concentrations of GT1b was not because of the toxicity.

The effect of GM1, GD1a, and GT1b on TNF-α release was similar to that on NO release (Fig. 5A). TNF-α released from GT1b (20 μg/ml)-treated microglia for 10 h was 497 ± 30 pg/ml whereas that from untreated cells was 233 ± 26 pg/ml. However, TNF-α detected from cells treated with GM1 (50 μg/ml) and GD1a (50 μg/ml) was similar to the control level; thus, 249 ± 28 and 161 ± 31 pg/ml TNF-α were detected, respectively.

However, in contrast to NO and TNF-α release, COX-2 expression was induced by GM1 and GD1a as well as GT1b (Fig. 5B). Microglia treated with GT1b (20 μg/ml), GM1 (50 μg/ml), or GD1a (50 μg/ml) for 12 h expressed COX-2 with similar intensities. Thus, these results suggest that GT1b could be a major type of gangliosides that were responsible for the effect of gangliosides on NO and TNF-α release. However, GM1 and GD1a together with GT1b could be involved in COX-2 expression.

Neuraminidase Abolished NO Release from Gangliosides, and GT1b-treated Microglia—We tested whether sialic acid residues of gangliosides and GT1b are important to activate microglia. For this, gangliosides and GT1b were treated with neuraminidase to remove sialic acid residues. Compared with
untreated gangliosides and GT1b, neuraminidase-treated gangliosides (desialylated GT1b) and neuraminidase-treated GT1b (desialylated GT1b) produced only 15.6 ± 1.9 and 26.0 ± 3.3% of NO, respectively (Fig. 6, A and B). Neuraminidase alone had no effect on NO release (data not shown). These results suggest that sialic acid residues of gangliosides and GT1b are important for inducing microglial activation.

**Gangliosides Induced Activation of Mitogen-activated Protein Kinases**—We studied whether gangliosides could activate MAPks because ERK and p38 mediated LPS- and Aβ-induced microglial activation (20, 34, 38). Tyrosine phosphorylation of ERK1/2 and JNK/SAPK was detected within 15 min and that of p38 was detected within 30 min after treatment of cells with 50 μg/ml gangliosides. The activities of ERK1/2 and JNK/SAPK were sustained up to 2 h whereas those of p38 decreased to the control level. It is noticeable that ERK was significantly activated, but JNK/SAPK and p38 were relatively weakly activated (Fig. 7). LPS treatment induced activation of three subtypes of MAPks as previously reported (20, 38). The pharmacological studies using inhibitors of ERK kinase (PD98059) and p38 (SB203580) showed comparable results to the activation pattern of MAPks. In the presence of 1 and 5 μM PD98059, NO release was reduced to 54.5 ± 8.9 and 45.6 ± 15.5%, respectively. However, in the presence of 10 and 50 μM of SB203580, NO release was 123.1 ± 15.7 and 120.8 ± 16.4% of that in the absence of SB203580 (Fig. 8A). PD98059 and SB203580 also showed a similar effect on NO release from GT1b-treated cells. In the presence of 5 μM PD98059, NO release was reduced to 43.5 ± 2.6%; however, in the presence of 10 and 50 μM SB203580, NO release was 104 ± 4.0 and 130.7 ± 1.5% of that in the absence of any inhibitor (Fig. 8B). These results suggest that ERK could function more importantly than p38 in ganglioside-induced microglial activation, although the function of JNK/SAPK could not be estimated in this study.

**Gangliosides Induced Activation of NF-κB**—The promoter regions of genes encoding iNOS, COX-2, and TNF-α have NF-κB-binding sites (24–26). Furthermore, it has been reported that both Aβ and LPS induced activation of NF-κB in microglia (23). Thus, we examined whether gangliosides could activate NF-κB. The electrophoresis mobility shift assay showed that gangliosides activated NF-κB within 15 min and that the activity was gradually increased up to 1 h (represented by arrows in Fig. 9A). In the presence of excess amounts of unlabeled oligonucleotides the shifted bands disappeared (Fig. 9A). To investigate which subunits of NF-κB were activated by gangliosides, we carried out super-shift assay using antibodies against p50 and p65. In the presence of p65 antibodies, a super-shifted band appeared (represented by an arrow head in Fig. 9B), and the intensity of the upper shifted band was reduced (represented by the lower arrow in Fig. 9B). However, the intensity of the lower shifted band was barely changed (represented by the lower arrow in Fig. 9B). In contrast, p50 antibodies neither induced any super-shifted band nor reduced the intensity of shifted bands (Fig. 9B). Thus, these results suggested that p65 could be a major subunit of NF-κB activated by gangliosides.

We further examined whether the activation of NF-κB was directly related to the induction of NO release. For this, N-acetyl cystein (NAC), a known NF-κB inhibitor (39, 40), was
treated with gangliosides or GT1b. NAC reduced NO release from both ganglioside- and GT1b-treated cells. In the presence of 1, 5, and 10 mM NAC, ganglioside-induced NO release was reduced to 59.6 ± 7.0, 33.4 ± 9.6, and 32.0 ± 3.5%, and GT1b-induced NO release was reduced to 85.0 ± 6.1, 1.7 ± 4.6, and −17.1 ± 5.0% (Fig. 10, A and B). Thus, these results suggest that gangliosides activate microglia via activation of NF-κB.

DISCUSSION

Gangliosides are found in neuronal cell membranes. Thus, gangliosides could be released from damaged neuronal cells to the extracellular space in injured brain. Several studies support this possibility; the amount of gangliosides in cerebrospinal fluid increases in neurodegenerative disease (41) and in HIV-infected brain (42). The results in this study showed that gangliosides induced expression of iNOS and COX-2 and release of TNF-α; all are markers of activated microglia. Furthermore, gangliosides produced these effects via activation of MAPKs and NF-κB. Thus, gangliosides released from damaged brain cells could act as a microglial activator.

The specific types of gangliosides showed a differential effect on microglial activation (Figs. 4 and 5). GT1b induced production of NO and TNF-α and expression of COX-2 whereas GM1 and GD1a induced COX-2 expression only. Differential functions of specific types of gangliosides have been reported in several cell types. GM3 but not GM1 inhibited autophosphorylation of the epidermal growth factor receptor (43). GM1 was also incorporated into Jurkat T cells and activated phospholipase C (54). However, gangliosides did not induce microglial activation via expression of other signaling pathway(s) that inhibit microglial activation. However, further studies should be done to clarify this hypothesis, because the intracellular signaling pathways activated by gangliosides have not been yet fully understood.

Gangliosides could produce their cellular functions in several ways. One way is through the specific binding of gangliosides to the surface receptors. Although receptors for each ganglioside have not been clearly elucidated, galectins have been suggested as receptors for GM1 because GM1 bound to galectin-1 and -3 but GM3 did not (49). The effect of gangliosides on cells could be potentiated by an increase in the number of receptors for ganglioside as well. The expression of receptors for gangliosides could be changed during the modulation of cellular properties. Galectin-1-like protein in macrophages was up-regulated in activation of these cells (50, 51), and galectin-3 in murine microglia was up-regulated by the granulocyte-macrophage colony-stimulating factor (52). The other way is through the incorporation into the plasma membrane. GM3 was incorporated into the plasma membrane and down-regulated CD4 molecules in T lymphocytes (22) and into modulated growth factor receptors in glial cells (53). GM1 was also incorporated into Jurkat T cells and activated phospholipase C (54). However, gangliosides did not induce microglial activation via expression of other glycosphingolipids. D-threo-PDMP (1-phenyl-2-decanoylamino-3-morpholino-1-propanol), a blocker of synthesis of all glycosphingolipids, did not inhibit gangliosides-induced NO, TNF-α, and COX-2 expression (data not shown).

The results in this study showed that gangliosides activated MAPKs and NF-κB in microglia (Figs. 7–10). MAPKs and NF-κB might be common pathways to mediate microglial activation because LPS and Aβ also activated MAPKs and NF-κB in microglia (20, 34, 38). However, some distinctive effects were detected between gangliosides and LPS. 1) JNK/SAPK

Fig. 9. Gangliosides activated NF-κB. Nuclear extracts were prepared from microglia treated with 50 μg/ml gangliosides for the indicated time periods. A, the NF-κB-specific oligonucleotide-protein complex was marked by an arrow. The excess amount of unlabeled oligonucleotides abolished the shifted bands (cold 30 and cold 60, respectively). B, for super-shift assay, antibodies against p50 and p65 were added to nuclear extracts from ganglioside-treated microglia for 30 min. The super-shifted band detected in the presence of p65 antibodies is marked by an arrow head. The electrophoresis mobility shift assay was carried out as described under “Experimental Procedures.”

Fig. 10. N-acetyl cysteine (NAC) inhibited NO release from ganglioside- or GT1b-treated microglia. Microglia were treated with 50 μg/ml gangliosides (A) or 10 μg/ml GT1b (B) for 48 h in the presence or absence of indicated amounts of NAC. Values were mean ± S.E. of three samples. *, p < 0.05; **, p < 0.01; ***, p < 0.001 as compared with NO release in the absence of NAC.
and p38 were less strongly activated by gangliosides than LPS whereas ERK was strongly activated by both gangliosides and LPS. 2) The inhibition of p38 reduced LPS-induced NO release (20) but neither gangliosides-, nor GT1b-induced NO release (Fig. 8). 3) In dose-response experiments, gangliosides and GT1b showed a bell-shaped effect on NO release in the range of 1 to 200 μg/ml, and 2 to 20 μg/ml, respectively. However, LPS linearly increased NO release in the range of 1 ng/ml to 1 μg/ml and did not show a bell-shaped effect at even higher concentrations (data not shown).

Although ERK and NF-κB could be common pathways involved in microglial activation, they might not be sufficient to induce NO and TNF-α release. GM1 and Aβ activated ERK and NF-κB as strongly as did gangliosides (data not shown). However, GM1 failed to produce NO and TNF-α release (Figs. 4 and 5), and Aβ had little effect on NO release (20). These results suggested that activation profiles of microglia could be different depending on stimulators and that gangliosides might activate other signaling pathways as well as ERK and NF-κB.

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