Cytotoxic effects of $G_M1$ ganglioside and amyloid $\beta$-peptide on mouse embryonic neural stem cells

Makoto Yanagisawa, Toshio Ariga and Robert K Yu

Institute of Molecular Medicine and Genetics and Institute of Neuroscience, Medical College of Georgia, Augusta, GA 30912, U.S.A.

Cite this article as: Yanagisawa M, Ariga T and Yu RK (2010) Cytotoxic effects of $G_M1$ ganglioside and amyloid $\beta$-peptide on mouse embryonic neural stem cells. ASN NEURO 2(1):art:e00029.doi:10.1042/AN20090063

ABSTRACT

AD [Alzheimer’s disease] is a neurodegenerative disease and the most common form of dementia. One of the pathological hallmarks of AD is the aggregation of extracellular A$s$ (amyloid $\beta$-peptides) in senile plaques in the brain. The process could be initiated by seeding provided by an interaction between $G_M1$ ganglioside and A$s$s. Several reports have documented the bifunctional roles of A$s$s in NSCs [neural stem cells], but the precise effects of $G_M1$ and A$\beta$ on NSCs have not yet been clarified. We evaluated the effect of $G_M1$ and A$\beta$-(1–40) on mouse NECs [neuroepithelial cells], which are known to be rich in NSCs. No change of cell number was detected in NECs cultured in the presence of either $G_M1$ or A$\beta$-(1–40). On the contrary, a decreased number of NECs were cultured in the presence of a combination of $G_M1$ and A$\beta$-(1–40). The exogenously added $G_M1$ and A$\beta$-(1–40) were confirmed to incorporate into NECs. The Ras–MAPK [mitogen-activated protein kinase] pathway, important for cell proliferation, was intact in NECs simultaneously treated with $G_M1$ and A$\beta$-(1–40), but caspase 3 was activated. NECs treated with $G_M1$ and A$\beta$-(1–40) were positive in the TUNEL [terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling] assay, an indicator of cell death. It was found that $G_M1$ and A$\beta$-(1–40) interacted in the presence of cholesterol and sphingomyelin, components of cell surface microdomains. The cytotoxic effect was found also in NSCs prepared via neurospheres. These results indicate that A$\beta$-(1–40) and $G_M1$ co-operatively exert a cytotoxic effect on NSCs, likely via incorporation into NEC membranes, where they form a complex for the activation of cell death signalling.

Key words: Alzheimer’s disease (AD), amyloid $\beta$-peptide (A$\beta$), apoptosis, $G_M1$ ganglioside, glycosphingolipid, neural stem cell.

INTRODUCTION

AD (Alzheimer’s disease), the most common form of dementia, is a neurodegenerative disease that manifests clinically as progressive memory loss and cognitive impairment. Pathologically, it is characterized by the appearance of senile plaques and neurofibrillary tangles and loss of neurons in the cerebral cortex (Ariga et al., 2008). Elucidation of the pathogenesis of AD, which is not yet fully understood, is an important subject for the development of therapies for this disease. One of the pathological hallmarks and possible causes of AD is the aggregation and accumulation of extracellular A$s$s [amyloid $\beta$-peptides] in the brain. A$s$s, which are produced from amyloid precursor protein by sequential endoproteolytic cleavages by $\beta$-secretase and $\gamma$-secretase, are originally monomeric, soluble and non-toxic, but become cytotoxic by aggregation and accumulation. Senile plaques seen in the brains of AD patients are mainly composed of the aggregated and accumulated A$s$s. The aggregation of A$s$s, a critical step in the onset of AD, has been proposed to be initiated by $G_M1$ ganglioside (Hayashi et al., 2004; Yanagisawa, 2007).

Gangliosides, sialic acid-containing glycosphingolipids, are ubiquitously expressed in vertebrate cells and body fluids, and are particularly abundant in the nervous system (Yu et al., 2009). In cells, gangliosides are localized primarily, although not exclusively, on the plasma membrane. Together with cholesterol and sphingomyelin, gangliosides are components of cell surface microdomains (glycosphingolipid-enriched microdomains, caveolae or lipid rafts). These specialized microdomain structures have been suggested to serve as important platforms for modulating cell adhesion and signal transduction. In AD brains, $G_M1$ ganglioside, one of the major brain gangliosides, is considered to bind to monomeric A$s$s and initiate their...
aggregation and accumulation by serving the role of 'seeding' (Hayashi et al., 2004; Yanagisawa, 2007). On the other hand, gangliosides are widely known to have neuroprotective effects (Svennerholm, 1994; Ledeen and Wu, 2002; Svennerholm et al., 2002). Certain gangliosides, including GM₁, have also been reported to inhibit Aβ-induced secretion of pro-inflammatory cytokines such as IL-1β (interleukin-1β), IL-6 and tumour necrosis factor-α, which are involved in the pathogenic events of AD (Ariga and Yu, 1999; Ariga et al., 2001a). Understanding the relationship between Aβ and GM₁ and their bidirectional effects is important to fully elucidate the pathogenesis of AD.

At present, there is still no well-established cure for AD. The strategy of using endogenous or transplanted NSCs (neural stem cells) to compensate for the neuronal loss in AD brains has been proposed. Because of their basic biological importance and latent clinical usefulness for treating a variety of neurodegenerative diseases (such as AD), NSCs (which are undifferentiated neural cells that are endowed with a high potential for proliferation and the capacity for self-renewal retaining the multipotency to differentiate into neuronal and glial cells) have been of great interest during the last two decades (Sugaya, 2003). In adult mammalian brains, NSCs are localized in the subventricular zone of the lateral ventricles and the subgranular layer of the dentate gyrus in the hippocampus. Nevertheless, neurogenesis in these areas does not compensate for neuronal loss in AD brains, implying the possibility that this impairment of neurogenesis, especially in the hippocampal dentate gyrus, is involved in the pathogenesis of AD. So far, several reports have documented the bifunctional roles of Aβs in NSCs in vitro; Aβs have neurogenic effects in some studies (Lopez-Toledano and Shelanski, 2004; Waldau and Shetty, 2008; Chen and Dong, 2009; Sothibundhu et al., 2009), but cytotoxic effects in other studies (Haughey et al., 2002a, 2002b; Millet et al., 2005; Calafiore et al., 2006; Waldau and Shetty, 2008). However, the effects of a combination of Aβ and GM₁ on NSCs have not yet been clarified. In the present study, we evaluated the effects of GM₁ and Aβ-(1–40), the isoform of 40 amino acid residues in length, on mouse NEC (neuroepithelial cell), which are known to be rich in NSCs (Fukuda et al., 2007).

MATERIALS AND METHODS

Materials

The GM₁ ganglioside used in the present study was isolated from human brain in our laboratories (Ledeen and Yu, 1982). Aβ-(1–40) was purchased from Bachem Americas (Torrance, CA, U.S.A.; catalogue number H–1194.0001; lot number 2500610). Tunicamycin, an inhibitor of N-linked glycosylation, was purchased from Sigma–Aldrich (St Louis, MO, U.S.A.).

NEC culture

NECs were prepared from telencephalons of mouse embryos (embryonic day 14.5) and cultured in N2-DMEM/F12 [N2-supplemented DMEM (Dulbecco’s modified Eagle’s medium)/Ham’s Nutrient Mixture F12] containing bFGF (basic fibroblast growth factor; Peprotech, Rocky Hill, NJ, U.S.A.) on dishes coated with poly-L-ornithine and fibronectin (Sigma–Aldrich) by the method described by Nakashima et al. (1999). Mice used for the cell preparation were treated according to the guidelines of the IACUC (Institutional Animal Care and Use Committee) of the Medical College of Georgia to minimize pain or discomfort. NECs cultured for 6 days were replated for treatment with GM₁ and/or Aβ-(1–40). For treatment of NECs, GM₁ was completely dried under a stream of nitrogen and then dissolved in N2-DMEM/F12 at 37 °C. Aβ-(1–40) dissolved in PBS and stored at −80 °C as a stock solution was directly added to N2-DMEM/F12 immediately before treatment of NECs; any procedures to induce the formation of the oligomeric or fibrillary form of Aβ-(1–40) were not performed. The NECs treated with GM₁ and/or Aβ-(1–40) were used for the experiments described below.

WST-8 assay

The number of cells cultured in the presence or absence of GM₁ and/or Aβ-(1–40) on poly-L-ornithine- and fibronectin-coated 96-well plates for 4 days was estimated by the WST-8 assay, a highly sensitive and reproducible method (Kanemura et al., 2002; Yu and Yanagisawa, 2007; Yanagisawa and Yu, 2009), using a Cell Counting Kit-8 (Dojindo, Kumamoto, Japan). The A₅₇₀ of WST-8-formazan produced by the dehydrogenase activity in the living cells was measured (reference: 650 nm) using a Benchmark Plus microplate spectrophotometer (Bio-Rad Laboratories, Hercules, CA, U.S.A.).

Cell staining

NECs cultured in N2-DMEM/F12 containing GM₁ and/or Aβ-(1–40) or FITC-Aβ-(1–40) [FITC-conjugated Aβ-(1–40); Bachem Americas] on chamber slides (Nalge Nunc International, Naperville, IL, U.S.A.) for 2 or 3 days were fixed in PBS containing 4% (w/v) paraformaldehyde and stained with biotin-Ctxb (biotin-conjugated cholera toxin B subunit), rat 401 anti-nestin monoclonal antibody (BD Biosciences, San Jose, CA, U.S.A.) or anti-β-III tubulin monoclonal antibody (Sigma–Aldrich). Biotin-Ctxb and monoclonal antibodies were detected with rhodamine-conjugated streptavidin (Jackson ImmunoResearch, West Grove, PA, U.S.A.) and Alexa Fluor® 488-conjugated anti-mouse IgG antibody (Invitrogen, Carlsbad, CA, U.S.A.) respectively. Nuclei were stained with Hoechst 33258 (Sigma–Aldrich). The stained NECs were photographed under a Nikon Eclipse TE300 fluorescent microscope (Nikon Instruments, Melville, NY, U.S.A.) equipped with a Magnafire digital CCD camera (charge-coupled device camera; Optronics, Goleta, CA, U.S.A.).
Effects of Gm1 and Aβ on neural stem cells

Western-blot analysis

Western-blot analysis was performed as previously described (Yanagisawa and Yu, 2009). As primary antibodies, anti-phospho-ERK (extracellular-signal-regulated kinase) monoclonal antibody (Cell Signaling Technology, Danvers, MA, U.S.A.), anti-ERK polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.), anti-p27kip1 monoclonal antibody (BD Biosciences), anti-β-actin monoclonal antibody (Sigma–Aldrich) and anti-caspase 3 rabbit monoclonal antibody (Cell Signaling Technology) were used. Horseradish-peroxidase-conjugated anti-mouse IgG antibody (GE Healthcare Life Sciences, Piscataway, NJ, U.S.A.) and anti-rabbit IgG antibody (GE Healthcare Life Sciences) were used as the secondary antibodies. Protein bands reacted with the antibodies were detected using WesternLightning Western Blot Chemiluminescence Reagent Plus (PerkinElmer Life and Analytical Sciences, Waltham, MA, U.S.A.).

RT–PCR (reverse transcription–PCR)

RT–PCR was performed as previously described (Ngamukote et al., 2007). The sequences of primer sets were as follows: 5′-TGAAGTGCTACATACAGAC-3′ and 5′-GGTATAGGCAAG-GGGGAAG-3′ for nestin; 5′-CCTACGTGACGAGAAACAG-3′ and 5′-CTGGGTTCTGTGCTCTGTTC-3′ for MAP2 (microtubule-associated protein 2); 5′-ACCACGTCTAGCCATTAC-3′ and 5′-TCCACACCTGTGCTGTA-3′ for GAPDH (glyceraldehyde-3-phosphate dehydrogenase).

TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling)

Apoptotic cells were detected with the TUNEL assay. In brief, cells were plated on to chamber slides and cultured in N2-DMEM/F12 containing Gm1 and Aβ-(1–40) in the presence of 5 ng/ml of bFGF for 3 days. Then, the cells were fixed in PBS containing 4% paraformaldehyde for 1 h at room temperature (approx. 25°C) and permeabilized in 0.1% sodium citrate containing 0.1% Triton X-100 for 2 min at 4°C. The cells were incubated with fluorescein-conjugated TUNEL reaction mixture (Roche Diagnostics, Mannheim, Germany) for 2 h at 37°C. Nuclei were stained with Hoechst 33258. Stained cells were photographed under a Nikon Eclipse TE300 fluorescent microscope equipped with a Magnafire digital CCD camera.

Gm1 binding assay

Physical interaction between Gm1 and Aβ-(1–40) was revealed by a Gm1 binding assay using Gm1-coated 96-well plates and FITC-Aβ-(1–40). In brief, polystyrene 96-well white plates (Nalge Nunc International) were coated with Gm1 by adding a methanolic solution of Gm1 (0, 1 or 10 nmol per well) and/or cholesterol (Sigma–Aldrich; 0 or 7.5 nmol per well) and sphingomyelin (Sigma–Aldrich; 0 or 7.5 nmol per well) to each well; the molar proportion of the lipids was 40:30:30 (Gm1/cholesterol/sphingomyelin) (Kakio et al., 2001). After the methanol solvent had been evaporated at 37°C, a blocking solution of PBS containing 1% BSA was added. Gm1 immobilized on the wells was incubated with Hanks buffered saline solution containing 5 μM of an FITC-Aβ-(1–40) solution in the dark for 1 h at room temperature. After washing, the fluorescent intensity of FITC-Aβ-(1–40) binding to Gm1 immobilized on the wells was measured using a Victor3 V multilabel plate reader (PerkinElmer Life and Analytical Sciences) equipped with a λex=485 nm filter and a λem=535 nm filter. To evaluate the efficiency of the assay, the polystyrene 96-well plates coated with Gm1 (0, 1, 10, 100 and 1000 pmol per well) were incubated with 1 μM of biotin-Ctxb and then 2 μg/ml of Cy2-conjugated streptavidin (Jackson ImmunoResearch).

NSC culture

NSCs were prepared in the form of neurospheres, floating clonal aggregates formed by NSCs in vitro, according to previously described methods but with slight modifications (Reynolds et al., 1992; Nakatani et al., 2010). In brief, single-cell suspensions prepared by striata of mouse embryos (embryonic day 14.5) by mechanical trituration were cultured in N2-DMEM/F12 containing 20 ng/ml bFGF and 20 ng/ml epidermal growth factor (Peprotech). Neurospheres formed after 1 week were collected for passage or further analyses. Confirmation of NSCs was performed by cell staining using a subcell control IgG (BD Biosciences) or anti-nestin monoclonal antibody.

RESULTS

First, we examined the number of NECs treated with low concentrations of Gm1 and Aβ-(1–40). As shown in Figure 1, lower concentrations of Gm1 (1, 5 or 10 μM) and/or Aβ-(1–40) (1 or 5 μM) have almost no effect on the NEC number. It has been reported that the effect of Aβ on NSCs is highly dependent on the concentration; monomeric Aβ showed no significant effect on proliferation and differentiation of adult mouse NSCs at lower concentrations, but inhibited the proliferation and neurogenesis at higher concentrations (Heo et al., 2007). Therefore we evaluated the effects of higher concentrations of Gm1 and Aβ-(1–40) by culturing NECs in the presence of 40 μM Gm1 and 10 μM Aβ-(1–40) on NECs. Neither 40 μM Gm1 nor 10 μM Aβ-(1–40) affected the NEC number (Figure 2). However, in NECs cultured in the presence of both 40 μM Gm1 and 10 μM Aβ-(1–40), a significant reduction in the cell number was detected (Figure 2). This result indicates that Aβ-(1–40) in the presence of Gm1 has a cytotoxic effect on NECs.

Generally, gangliosides exogenously added to tissue culture have been expected to be incorporated into the cell
membranes through the hydrophobic ceramide residues. However, it is unclear whether exogenously added GM1 and Aβ-(1–40) were incorporated into NECs. Taking advantage of barely detectable quantities of GM1 expressed in NECs (Yanagisawa et al., 2006a), we evaluated the incorporation of GM1 into the NECs by staining with Ctxb, a probe recognizing GM1 and other related gangliosides. Simultaneously, Aβ-(1–40) distribution was examined by culturing NECs with FITC-Aβ-(1–40). As shown in Figure 3, GM1-treated NECs showed a strong reactivity to Ctxb. In addition, a strong FITC signal was found in NECs treated with FITC-Aβ-(1–40). These results indicate that exogenously added GM1 and Aβ-(1–40) were efficiently incorporated into NECs. In the NECs simultaneously treated with GM1 and Aβ-(1–40), however, there was no difference in their fluorescence intensities. Therefore it was confirmed that the co-operative effect of GM1 and Aβ-(1–40) on the number of NECs is mediated after the incorporation into NECs.

We then analysed the molecular mechanism underlying the reduction of the NEC number by GM1 and Aβ-(1–40). The Ras–MAPK (mitogen-activated protein kinase) pathway is known to be essential for the proliferation of NECs (Yanagisawa et al., 2005). In NECs treated with GM1 and/or Aβ-(1–40), however, bFGF-induced activation of the Ras–MAPK pathway was intact (Figure 4A). A cyclin-dependent kinase inhibitor, p27Kip1, which has been suggested to be up-regulated by ganglioside stimulation and involved in ganglioside-induced inhibition of neural cell proliferation (Nakatsuji and Miller, 2001), was not up-regulated in NECs treated with GM1 and/or Aβ-(1–40) (Figure 4B). These results indicate that GM1 and Aβ-(1–40) did not inhibit the proliferation of NECs, at least not at these signalling steps. Generally, the process of differentiation decreases the proliferation rate of stem cells. In fact, it has been reported that a low concentration of Aβ-(1–42) enhances neuronal differentiation of adult NSCs (Heo et al., 2007). In NSCs treated with GM1 and/or Aβ-(1–40), however, expression of nestin, a marker protein of NSCs, was not down-regulated (Figures 5A and 5C). The number of cells positive for β-IId tubulin, a marker protein of mature neurons scarcely found in untreated NECs, was not increased in GM1- and Aβ-(1–40)-treated NECs (Figure 5B). MAP2, a marker protein of immature and mature neurons, was not up-regulated in GM1- and Aβ-(1–40)-treated NECs (Figure 5C). These results indicate that the NECs treated with GM1 and/or Aβ-(1–40) were not differentiated. On the other hand, it has been reported that Aβ-(1–40) and Aβ-(1–42) induce apoptotic cell death in substantia nigra/neuroblastoma cell line cells (Le et al., 1995) and adult NSCs (Heo et al., 2007) respectively. As well, in NECs treated with GM1 and/or Aβ-(1–40), caspase 3 (a critical executioner of apoptosis or programmed cell death signalling) was synergistically activated (Figure 6A). It was confirmed that a number of NECs treated with GM1 and Aβ-(1–40) were positive for the TUNEL assay, an indicator of cell death accompanied by DNA fragmentation (Figures 6B and 6C). These results suggest that the reduction of NEC number by GM1 and Aβ-(1–40) was
caused, at least in part, by activation of the cell death process, but not by the inhibition of proliferation or the induction of differentiation, after incorporation into the NECs.

Figure 5 Neural lineage marker expression in NECs treated with GM1 and Aβ-(1–40)
Expression of (A) nestin (a marker protein of neural stem cells) and (B) β-III tubulin (a marker protein of mature neurons) in NECs treated with GM1 (0 or 40 μM) and Aβ-(1–40) (0 or 10 μM) for 3 days was analysed by cell staining. Nuclei were stained with Hoechst 33258. (C) Expression of nestin and MAP2 (a marker gene of immature and mature neurons) in NECs treated with GM1 (0 or 40 μM) and Aβ-(1–40) (0 or 10 μM) for 2 days was analysed by RT-PCR. ‘G3PDH’ indicates control GAPDH. ‘-RT’ indicates negative controls without reverse transcription.

Figure 6 Apoptosis of NECs treated with GM1 and Aβ-(1–40)
(A) Activation of caspase 3 (a critical executioner of apoptosis or programmed cell death signalling) in NECs treated with GM1 (0 or 40 μM) and Aβ-(1–40) (0 or 10 μM) in the presence of bFGF (5 ng/ml) for 2 days was analysed by Western blotting. An inhibitor of N-linked glycosylation, tunicamycin (1 μg/ml for 10 h), was used as a positive control to activate stress-mediated cell death signalling. (B) Apoptotic cells in NECs treated with GM1 (0 or 40 μM) and Aβ-(1–40) (0 or 10 μM) in the presence of bFGF (5 ng/ml) for 3 days were detected with the TUNEL assay. Nuclei were stained with Hoechst 33258. (Q) The proportion of TUNEL-positive cells in NECs treated with or without GM1 and Aβ-(1–40).
Our previous study using surface plasmon resonance and a liposome capture method showed that \( \text{G}_\text{M1} \) directly bound to \( \text{A}\beta \) with high affinity (Ariga et al., 2001b). This finding suggests that there is a possibility that sequestering \( \text{A}\beta-(1–40) \) with \( \text{G}_\text{M1} \) can inhibit the cytotoxic effect. To evaluate this possibility, we pre-incubated \( \text{A}\beta-(1–40) \) with \( \text{G}_\text{M1} \) to allow their binding and then cultured NECs in the presence of this pre-incubated mixture. However, the cytotoxic effect of \( \text{A}\beta-(1–40) \) was not inhibited by pre-incubation with \( \text{G}_\text{M1} \). 10 \( \mu \)M of \( \text{A}\beta-(1–40) \) pre-incubated with 40 \( \mu \)M of \( \text{G}_\text{M1} \) drastically reduced the NEC number (Figure 7). Thus we confirmed the physical interaction between \( \text{G}_\text{M1} \) and \( \text{A}\beta-(1–40) \) by the \( \text{G}_\text{M1} \) binding assay. First, we confirmed the efficiency of the assay by detecting the interaction between \( \text{G}_\text{M1} \) and the well-known ligand, Ctxb. As shown in Figure 8(A), this assay could dose-dependently detect binding of Ctxb to \( \text{G}_\text{M1} \) immobilized on 96-well plates. Then, we analysed the physical interaction between \( \text{G}_\text{M1} \) and FITC-A\( \beta-(1–40) \) using this assay. As shown in Figure 8(B), FITC-A\( \beta-(1–40) \) strongly interacts with \( \text{G}_\text{M1} \) only in the presence of cholesterol and sphingomyelin, components of cell surface microdomains, as reported by Kakio et al. (2001); the interaction between \( \text{G}_\text{M1} \) and FITC-A\( \beta-(1–40) \) was relatively weak in the absence of cholesterol and sphingomyelin. This result suggests that \( \text{G}_\text{M1} \) itself could not sequester A\( \beta-(1–40) \) in vitro and reduce the cytotoxicity. So far, the existence of cell surface microdomains, which are rich in cholesterol and sphingomyelin, has been confirmed in NECs (Yanagisawa et al., 2004). It is expected that exogenously added \( \text{G}_\text{M1} \) is incorporated into NECs and distributed to the cell surface microdomains. Binding of A\( \beta-(1–40) \) to NECs and activation of cell death signalling may occur in \( \text{G}_\text{M1} \)-positive microdomains.

As we have described, NECs are known to be rich in NSCs. However, NECs are still a heterogeneous cell population containing a few differentiated cells. To confirm the cytotoxic effect of \( \text{G}_\text{M1} \) and A\( \beta-(1–40) \) on NSCs, we prepared NSCs from striata of mouse embryos in the form of neurospheres.

**DISCUSSION**

In the present study, we clearly showed that \( \text{G}_\text{M1} \) and A\( \beta-(1–40) \) co-operatively have cytotoxic effects on NECs. This finding suggests the possibility that a combination of A\( \beta \) and \( \text{G}_\text{M1} \) is cytotoxic to not only neurons, but also NSCs in AD brains. In adult mammalian brains, \( \text{G}_\text{M1} \) is well known to be highly expressed (Ngamukote et al., 2007; Yu et al., 2009). The expression of \( \text{G}_\text{M1} \) may be involved in the impairment of neurogenesis in the AD brains. It has been reported that neural precursor cells isolated from post-mortem AD patient brains exhibit a severe reduction in number during culture (Lovell et al., 2006). A\( \beta \) and \( \text{G}_\text{M1} \) may be involved in this reduction in the...
number of neural precursor cells. In pathological conditions, however, the involvement of gangliosides other than GM1 should also be considered. For instance, Ab s have been reported to bind to rat phaeochromocytoma, PC12 cells; the binding does not seem to be with GM1, but rather with fucosylated GM1, which is expressed (Yanagisawa et al., 2006b). In addition, an in vitro study using surface plasmon resonance clarified that a-series gangliosides (GQ1b and GT1a) and b-series gangliosides (GQ1b, GT1b, GD2, and GD1a) have higher affinities for Ab than GM1 (Ariga et al., 2001b). More importantly, a recent analysis of GD3 synthase-knockout mice cross-bred with AD model mice has suggested that the bona fide gangliosides initiating Ab aggregation in the AD brains are b-series gangliosides, but not GM1 (Bernardo et al., 2009). Therefore other gangliosides expressed in NECs such as GD3, GQ1b and GT1a (Yanagisawa et al., 2004; Ngamukote et al., 2007) may also increase the neurotoxic effects of Ab on NSCs in the pathological condition of AD. Although there have been many reports so far, the functional roles of Ab s on NSCs are still controversial, perhaps because of the ganglioside species differentially expressed in these cells. Evaluation of the effects of exogenous GM1, on neurogenesis and pathogenesis of AD under pathological conditions, for instance using AD model mice (Jankowsky et al., 2001), will be an interesting and fruitful subject for future studies. These studies to understand the roles of GM1 and Ab on NSCs in AD may contribute to the development of new regenerative therapies for this disease.

ACKNOWLEDGEMENTS

We are grateful to Dr Fung-Chow Chiu, Dr Chandramohan Wakade and Dr Yusuke Suzuki (Medical College of Georgia) for their technical help.

FUNDING

This work was supported by USPHS grants [grant numbers NS11853, NS26994 and AG027199]; a grant from the Children’s Medical Research Foundation (Chicago, IL, U.S.A.); and a start-up fund awarded to M.Y. from Medical College of Georgia.

REFERENCES

Ariga T, Yu RK (1999) GM1 inhibits amyloid β-protein-induced cytokine release. Neurochem Res 24:219–226.

Ariga T, Kiso M, Hasegawa A, Miyatake T (2001a) Gangliosides inhibit the release of interleukin-1β in amyloid β-protein-treated human monocytic cells. J Mol Neurosci 17:371–377.

Ariga T, Kobayashi K, Hasegawa A, Kiso M, Ishida H, Miyatake T (2001b) Characterization of high-affinity binding between gangliosides and amyloid β-protein. Arch Biochem Biophys 388:225–230.

Ariga T, McDonald MP, Yu RK (2008) Role of ganglioside metabolism in the pathogenesis of Alzheimer’s disease – a review. J Lipid Res 49:1157–1175.

Bernardo A, Harrison FE, McCord M, Zhao J, Bruchey A, Davies SS, Jackson Roberts II L, Mathews PM, Matsuoka Y, Ariga T, Yu RK, Thompson R, McDonald MP (2009) Elimination of GD3 synthase improves memory and reduces amyloid-β plaque load in transgenic mice. Neurobiol Aging 30:1777–1791.
Calafiori M, Battaglia G, Zapala A, Trovato-Salinaro E, Caraci F, Caruso M, Vancheri C, Sortino MA, Nicolotti F, Copani A (2006) Progenitor cells from the adult mouse brain acquire a neuronal phenotype in response to β-amyloid. Neurobiol Aging 27:606–613.

Chen Y, Dong C (2009) Al40 promotes neuronal cell fate in neural progenitor cells. Cell Death Differ 16:386–394.

Fukuda S, Abematsumori H, Mori H, Yanagisawa M, Kagawa T, Nakashima K, Yoshimura A, Taga T (2007) Potentiation of astrogenesis by STAT3-mediated activation of bone morphogenetic protein-Smad signaling in neural progenitor cells. J Neurochem 100:212–226.

Haughey NJ, Liu D, Nath A, Borchard AC, Mattson MP (2002a) Disruption of neurogenesis in the subventricular zone of adult mice, and in human cortical neural precursor cells in culture, by antibody against β-amyloid: implications for the pathogenesis of Alzheimer’s disease. Neuronomal Med 1:125–135.

Haughey NJ, Nath A, Chan SL, Borchard AC, Rao MS, Mattson MP (2002b) Disruption of neurogenesis by antibody β-peptide, and perturbed neural progenitor cell homeostasis, in models of Alzheimer’s disease. J Neurochem 83:1509–1524.

Hayashi H, Kimura N, Yamaguchi H, Hasegawa K, Yokoseki T, Shibata M, Yamamoto N, Michikawa M, Yoshikawa Y, Terao K, Matuszaki K, Lemere CA, Seikoe DJ, Naiki H, Yanagisawa K (2004) A seed for Alzheimer amyloid in the brain. J Neurosci 24:4894–4902.

Heo C, Chang KA, Choi HS, Kim HS, Kim S, Liew H, Kim JA, Yu E, Ma J, Suh YH (2007) Effects of the monomeric, oligomeric, and fibrillar Al42 peptides on the proliferation and differentiation of adult neural stem cells from subventricular zone. J Neurochem 102:493–500.

Jankowsky JL, Slunt HH, Ratovitski T, Jenkins NA, Copeland NG, Borchelt DR (2001) Co-expression of multiple transgenes in mouse CNS: a comparison of strategies. Biolog Eng 17:157–165.

Kakio A, Nishimoto SI, Yanagisawa K, Koizumi Y, Matuszaki K (2001) Cholesterol-dependent formation of GMI ganglioside-bound amyloid β-protein, an endogenous seed for Alzheimer amyloid. J Biol Chem 276:24985–24990.

Kanemura Y, Mori H, Kobayashi S, Islam O, Kodama E, Yamamoto A, Nakashiba Y, Aita N, Yamazaki M, Okano H, Hara M, Miyake J (2002) Evaluation of in vitro proliferative activity of human fetal neural stem/progenitor cells using indirect measurements of viable cells based on cellular metabolic activity. J Neurosci Res 69:869–879.

Liu W, Cao J, Liu J, Tao F, Xie JQ, Yang L, Smith RG, Lynn BC, Markesbery WR (2006) Isolation of neural precursor cells from Alzheimer’s disease and aged control postmortem brain. Neurobiol Aging 27:909–917.

Millet P, Lages CS, Haik S, Nowak E, Allemand I, Granotier C, Boussin FD (2005) Amyloid-β-peptide triggers Fas-independent apoptosis and differentiation of neural progenitor cells. Neurobiol Dis 19:57–65.

Nakashima K, Wiese S, Yanagisawa M, Arakawa H, Kimura N, Hisatsune T, Yoshida K, Kishimoto T, Sendtner M, Taga T (1999) Developmental requirement of gp130 signaling in neuronal survival and astrocyte differentiation. J Neurosci 19:5429–5434.

Nakatani Y, Yanagisawa M, Suzuki Y, Yu RK (2010) Characterization of G3 ganglioside as a novel biomarker of mouse neural stem cells. Glycobiology 20:78–86.

Nakatsuji Y, Miller RH (2001) Selective cell-cycle arrest and induction of apoptosis in proliferating neural cells by ganglioside GM3. Exp Neurol 166:290–299.

Nogamukote S, Yanagisawa M, Ariga T, Ando S, Yu RK (2007) Developmental changes of glycosphingolipids and expression of glycoproteins in mouse brains. J Neurochem 103:2327–2341.

Reynolds BA, Tetzlaff W, Weiss S (1992) A multipotent EGF-responsive striatal embryonic progenitor cell produces neurons and astrocytes. J Neurosci 12:4565–4574.

Sotthibundhu A, Li OX, Thangnipon W, Coulson EI (2009) Ap1–42 stimulates adult SVZ neurogenesis through the p75 neurotrophin receptor. Neurobiol Aging 30:1975–1983.

Sugaya K (2003) Neuroreplacement therapy and stem cell biology under disease conditions. Cell Mol Life Sci 60:1891–1902.

Svennerholm L (1994) Gangliosides – a new therapeutic agent against stroke and Alzheimer’s disease. Life Sci 55:2125–2134.

Svennerholm L, Brane G, Karlsson I, Lekman A, Ramstrom I, Wikkelso C (2002) Alzheimer disease – effect of continuous intracerebroventricular treatment with GM1 ganglioside and a systematic activation programme. Dementia Geriatr Cogn Disord 14:128–136.

Waldau B, Shetty AK (2008) Behavior of neural stem cells in the Alzheimer brain. Cell Mol Life Sci 65:2372–2384.

Yanagisawa K (2007) Role of gangliosides in Alzheimer’s disease. Biochim Biophysics Acta 1768:1943–1951.

Yanagisawa M, Yu RK (2009) O-linked β-N-acetylglucosaminylolation in mouse embryonic neural precursor cells. J Neurosci Res 87:3535–3545.

Yanagisawa M, Nakamura K, Taga T (2004) Roles of lipid rafts in integrin-dependent adhesion and gp130 signalling pathway in mouse embryonic neural precursor cells. Gene Cells 9:801–809.

Yanagisawa M, Nakamura K, Taga T (2005) Glycosphingolipid synthesis inhibitor represses cytokine-induced activation of the Ras-MAPK pathway in embryonic neural precursor cells. J Biochem (Tokyo) 138:285–291.

Yanagisawa M, Ariga T, Yu RK (2006a) Cholera toxin β subunit binding does not correlate with GM1 expression: a study using mouse embryonic neural precursor cells. Glycobiology 16:190–222.

Yanagisawa M, Ariga T, Yu RK (2006b) Fucosyl-GM1 expression and amyloid-β protein accumulation in PC12 cells. J Neurosci Res 84:1343–1349.

Yu RK, Yanagisawa M (2007) Glycosignaling in neural stem cells: involvement of glycoconjugates in signal transduction modulating the neural stem cell fate. J Neurochem 103 (Suppl. 1):39–46.

Yu RK, Nakatani Y, Yanagisawa M (2009) Role of glycosphingolipid metabolism in the developing brain. J Lipid Res 50:5440–5455.