Modified Eagle's Medium containing 1.2 mg/mL $NaHCO_3$, 110 μg/mL pyruvic acid, 25 μg/mL streptomycin, and 50 U/mL penicillin (mDMEM). After bisecting the brains along the central fissure, the meninges, hippocampi, and other subcortical structures were carefully removed, and the cerebral cortices were rinsed in culture medium (CM: mDMEM with 5% fetal calf serum) and minced into small pieces (<1 mm^3). The tissue pieces were digested at 32°C for 30 min in PBS containing 1.5 U/mL papain (Worthington Biochemical Corporation, Lakewood, NJ), 0.1 mg/mL DNase I (Roche Diagnostics, Japan), 0.2 mg/mL cysteine, 0.2 mg/mL albumin, and 5 mg/mL glucose. Cells were dissociated gently by passing the mixture several times through a disposable pipette, and then the mixture was centrifuged three times in CM at 800 rpm for 5 min at 32°C. For semiquantitative RT-PCR and Western Blotting experiments, cells were plated at 4.2×10^5 cells/cm^2 onto culture dishes coated with 0.125% polyethylenimine. All cultures were maintained at 37°C in a humidified chamber containing 95% ambient air and 5% CO_2. A half volume of culture supernatant was replaced with prewarmed ($\sim$37°C) CM once per week.

Rat primary cerebral cortical cultures prepared according to the above protocol consisted mainly of neurons ($>90\%$) with some astrocytes. We previously showed that these neuronal cells have complicated interactions with glia and other neurons and make synaptic connections with other neurons similar to those *in vivo* (Negishi *et al.*, 2002).

Rat Astrocyte Cultures

After 14 days, cerebral cortical cells were dissociated with 0.025% trypsin (Invitrogen) and washed several times in CM. Proliferating type-1 astrocytes were quickly selected from this suspension. After one subculturing, cells were plated at 2.0×10^4 cells/cm^2 onto uncoated 4-well LAB-TEK chamber slides (Nalge Nunc, Tokyo, Japan) for immunocytochemical studies. Cells were also plated at 4.2×10^5 cells/cm^2 in CM onto uncoated culture dishes for semiquantitative RT-PCR and Western Blotting experiments. A half volume of culture supernatant was replaced with prewarmed CM once per week (Negishi *et al.*, 2003).

Aβ Treatment

For semiquantitative RT-PCR and Western Blotting experiments, synthetic human Aβ peptides, Aβ1-40 (Aβ40) and Aβ1-42 (Aβ42) (Bachem, Torrance, CA), were dissolved in 100% DMSO, then diluted in CM (final concentration: 0.45% DMSO). These Aβ40 and Aβ42 solutions (5 μM, not preaggregated) were ultimately added to primary cortical cultures and astrocyte cultures, which were incubated for 3 h and 24 h at 37°C. For immunocytochemistry, Aβ peptides were dissolved in 100% DMSO, then diluted in CM without serum (final concentration: 0.45% DMSO). These Aβ solutions (10 nM, not preaggregated) were ultimately added to astrocyte cultures, which were incubated for 3 h at 37°C.

Semiquantitative RT-PCR

To make primers for LGI1, LGI3, and LGI4, we used the translated basic local alignment search tool nucleotide (tBLASTn) algorithm to compare known human LGI1 (NM005097), LGI3 (AF467956), and LGI4 (NM139284) sequences with rat LGI1 (NM145769), LGI3 (XM224337), and LGI4 (NM199499) sequences, respectively, contained within the NCBI expressed sequence tag (EST) database (http://www.ncbi.nlm.nih.gov).

Total cellular RNAs from untreated and Aβ-treated cell cultures were isolated using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). Single-stranded cDNAs were prepared via reverse transcription of a reaction mixture of total RNA (1 μg in 20 μL of reaction mixture), Oligo $(dT)_{12-18}$ primers, and Superscript™II (Invitrogen). PCR amplification was performed using Thermo-Start™ (Abgene) and the following primers:

lgi1 (+), 5′-GAAACCAGCGAAGCCAAAATGCCC-3′;
lgi1 (−),5′-GCGTGAATGATCTGGGTGCCTGAA-3′;
lgi3-1 (+), 5′-ATGGCCCGGCTACGAGCCAGGCG-3′;
lgi3-2 (−), 5′-CCAGTGGTAAATATATGAGCCA-3′;
lgi4 (+),5′-CATTCTGTTGTTCCTGTTGGCCTG-3′;
lgi4 (−),5′-TAGTAGTGGCTGGAAGACATGGGA-3′;
β-actin (+), 5′-ATGGATGACGATATCGCTG-3′;
β-actin (−), 5′-ATGAGGTAGTCTGTCAGGT-3′.

PCR products were electrophoresed in 1.5% agarose gels, and gels were subsequently stained with ethidium bromide. Densitometric analyses of gels were carried out with Quantity One Software (PDI Inc., NY, USA). We examined two independent cell preparations ($N = 6$) and duplicated each experiment.

Cloning and Sequence Analysis

To identify the sequence of F344 rat LGI3, we also used the tBLASTn algorithm to compare known human LGI3 (AF467956) sequences with predicted rat LGI3 (XM224337) sequences. Once identified, this sequence was used to aid in sequencing the rat LGI3 gene.

Adult rats were deeply anesthetized and their brains were carefully removed. The cerebral cortex, cerebellum, and rhinencephalon were dissected free from the diencephalon and brain stem. The diencephalon and brain stem were discarded, and the cerebral cortex was either combined with the cerebellum and rhinencephalon for RT-PCR, or was used alone for sequence computation. Total RNA was isolated from the cerebral cortices using TRIzol Reagent (Invitrogen). Single-stranded cDNAs were prepared via reverse transcription of a reaction mixture of total RNA (1 μg in 20 μL of reaction mixture), Oligo $(dT)_{12-18}$ primers, and Superscript™II (Invitrogen). PCR amplification was performed using Thermo-Start™ (Abgene,

UK) and the following primers:

lgi3-1 (+), 5′-ATGGCCCGGCTACGAGCCAGGCG-3′;
lgi3-1 (−), 5′-TTAGCCTCCCTCTAGGCACTAAG-3′;
lgi3-2 (+), 5′-CACCATCCTCAAGTGGGACTATGT-3′;
lgi3-2 (−), 5′-CCAGTGGTAAATATATGAGCCA-3′;
lgi3-3 (+), 5′-CTGGCACTGGGCAGTGA-CTTCTCCTTC-3′;
lgi3-3 (−), 5′-TTAGCCTCCCTCTAGGCACTAAGGT-3′.

The PCR product was cloned using a TOPO TA Cloning® Kit (Invitrogen), and the sequence of rat LGI3 was determined with an ABI PRISM™ 377 DNA Sequencer (Perkin Elmer, Boston, MA, USA).

Molecular Modeling of LGI3

We used the Molecular Operating Environment system (MOE; Ryoka Systems Inc., Japan) to construct the deduced structure of LGI3 protein.

Production of the Specific Antibody Against LGI3

The LGI3 peptide CSRTQKQFVAQGEVTQVP (TA142) was chemically synthesized by the Peptide Institute (Minoh-shi, Osaka, Japan) for use as an antigen to produce antibodies. TA142 was synthesized based on amino acid sequence 405–421of rat LGI3; its amino sequence completely matched that of human LGI3. Antibody against LGI3 was prepared by injecting two rabbits with TA142 coupled to keyhole limpet hemocyanin, mixed with RIBI adjuvant system R-730 (RIBI Immunochem Research, NH). Antibody against TA142 was purified from the immune rabbit serum by two-step chromatography: absorption with TA142-coupled sepharose (MBL, Japan) and then affinity column chromatography with TA142-coupled sepharose. Because purified anti-TA142 antibodies from the two rabbits resulted in similar immunoreactivity, we used only one of these antibodies for the Western Blotting, immunocytochemical, and immunohistochemical experiments.

Western Blot Analyses

For Western Blotting, the following antibodies were used: rabbit polyclonal anti-LGI3 (anti-TA142; MBL, Japan); mouse monoclonal antiglial fibrillary acidic protein (GFAP) (6F2; DAKO, Denmark); goat polyclonal antiapolipoprotein E (ApoE) (APO-E; Chemicon, Temecula, CA); and mouse monoclonal anti-β-actin (AC15; Sigma, St. Louis, MO).

To extract total cellular proteins from the cultured cells, the cells were bathed in a solution containing 9.85 mg/mL Tris-HCl, 0.774 mg/mL ethylenediaminetetraacetic acid (EDTA), 0.348 mg/mL ammonium persulfate, 0.5% (v/v) Triton X-100, and 2.3% (w/v) SDS in PBS. Total proteins were isolated by centrifugation, adjusted to 20 μg, then subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE with 10% acrylamide gel). Separated proteins were blotted onto polyvinylidene fluoride membranes (Immobilon P, Millipore, Bedford, MA). The membranes

were blocked with 5% nonfat dried milk in 20 mM PBS (pH 7.0) and 0.1% Tween-20 overnight at 4°C, then incubated with primary antibodies (TA142, 1:4000; 6F2, 1:5000; APO-E, 1:2000; AC15, 1:20000) for 1 h at room temperature. They were then incubated with horseradish peroxidase-conjugated goat antimouse IgG, mouse antirabbit IgG, or rabbit antigoat IgG (1:6000, Jackson Immunoresearch Laboratories, West Grove, PA) for 1 h at room temperature. Immunoreactive elements were visualized using enhanced chemiluminescence (ECLplus, Amersham, UK). We made up three independent cell preparations ($N = 6$) and duplicated each experiment. To confirm the specificity of anti-TA142 antibody, the primary antibody solution was preabsorbed with 50 $\mu g/\mu L$ of TA142 antigen peptide.

Data Analyses

Statistical analyses were performed by using one-way ANOVAs followed by the Fisher's posthoc test. Data are shown as means ± SD.

Immunocytochemistry

Cells plated on chamber slides were fixed with 4% paraformaldehyde at room temperature. After permeabilization with 0.01% Triton X-100, cells were incubated in primary antibody solution overnight at 4°C. For immunocytochemistry, the following antibodies were used: anti-TA142 (1:500), 6F2 (1:100), and mouse monoclonal anti-Aβ (4G8, 1:2000; Signet, Dedham, MA). Antibody 4G8 is specific against human Aβ, and under normal conditions, rarely recognizes rodent endogenous Aβ. Following brief washes with buffer, the cells were then sequentially incubated with Alexa 488-conjugated goat antimouse IgG (1:1000; Molecular Probes), Alexa 568-conjugated goat antirabbit IgG (1:1000; Molecular Probes), and DAPI (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA) for 30 min at room temperature. The slides were examined with a digital eclipse C1 confocal microscope (NIKON, Japan).

RESULTS

LGI Gene Expression During Aβ Treatment

Aβ has been shown to increase the expression of Lib, a gene that encodes a type I transmembrane protein harboring LRRs, in rat astrocytes (Satoh *et al.*, 2002). This finding prompted us to investigate whether Aβ also influences the expression of the LGI family of genes in cultured neural cells. Aβ did not influence LGI expression in primary cerebral cortical cultures (data not shown). This was true regardless of the type of Aβ species used: neither Aβ40 nor Aβ42 affected LGI family expression in cortical cultures (data not shown). In contrast, Aβ increased the expression of LGI3, but not LGI1 or LGI4, in astrocyte cultures after 3 h of Aβ treatment [Fig. 1(A)]. The Aβ-associated increase in LGI3 expression was statistically significant [Fig. 1(B)]. After 24 h, however, LGI3 expression dropped to almost that of the control (data not shown).

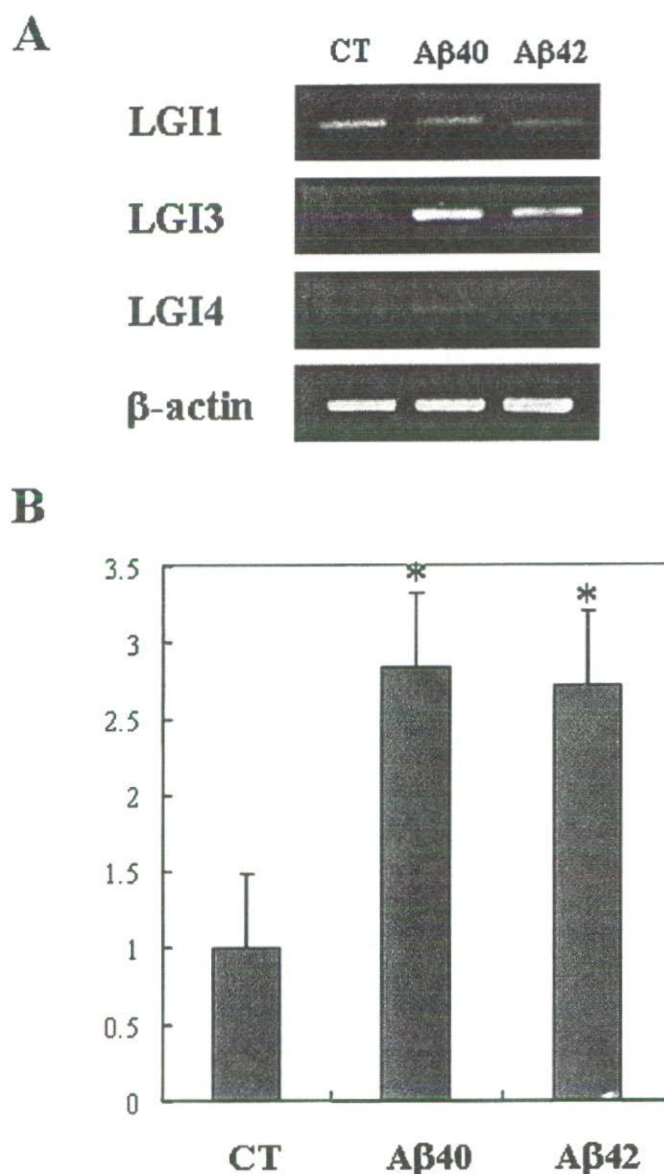

Fig. 1. (A) Expression patterns of rat LGI mRNA in rat astrocyte cultures treated with Aβ. Expression was assessed with semiquantitative RT-PCR (see Materials and Methods). β-actin primers were used for normalization of RNA concentrations, since β-actin is expressed in neural cells. Aβ induced only LGI3 gene expression but not LGI1 or LGI4 expression. In this experiment, Aβ40 and Aβ42 were diluted in culture medium to a final concentration of 5 μM. (B) Rat LGI3 mRNA levels in rat astrocyte cultures treated with either Aβ40 or Aβ42. All data were normalized according to β-actin mRNA levels (control group, CT). Values are means ± SD. $^{*}p < 0.02$. CT—control rat astrocyte cultures contained the same concentration of DMSO found in the Aβ treatment solutions; Aβ40—Aβ40 treatment; Aβ42—Aβ42 treatment.

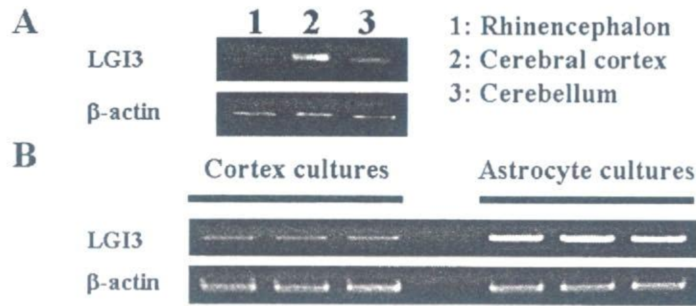

Fig. 2. Rat LGI3 mRNA expression patterns. (A) Rat brain. (B) Rat primary cerebral cortical cultures and rat astrocyte cultures. Expression was assessed with semiquantitative RT-PCR. As before, β-actin primers were also used for normalization of mRNA levels in these experiments. In rat brain, LGI3 mRNA was most abundantly expressed in the cerebral cortex (A). The expression of rat LGI3 mRNA in astrocyte cultures was much greater than that in primary cerebral cortical cultures (B).

LGI3 Expression in Rat Brain and Cultured Rat Neural Cells

First, we investigated the expression of LGI3 in rat brain to determine whether LGI3 is differentially expressed across the brain [Fig. 2(A)]. To evaluate the degree of LGI3 expression, we also measured the expression of β-actin (normalization gene), since β-actin is widely expressed in the brain [Fig. 2(A)]. LGI3 was expressed in the rhinenchephalon, cerebral cortex, and cerebellum [Fig. 2(A)]. Of these three regions, we observed the most robust LGI3 expression in the cerebral cortex, followed by the cerebellum [Fig. 2(A)]. Expression in the rhinencephalon was very weak compared to that in the cerebral cortex [Fig. 2(A)]. β-actin was equally expressed throughout the brain [Fig. 2(A)].

Second, we investigated the expression of LGI3 in cultured neuronal and glial cells to determine whether LGI3 is differentially expressed in various neural cell types [Fig. 2(B)]. The rat primary cerebral cortical cultures mainly consisted of neurons (>90%), whereas the rat astrocyte cultures consisted entirely of astrocytes (see Materials and Methods). Although LGI3 expression was observed in both cortical and astrocyte cultures, expression was greater in the astrocyte cultures [Fig. 2(B)].

Nucleotide and Amino Acid Sequence Comparisons and Modeling of LGI3 Protein Structure

To verify whether LGI3 produced by RT-PCR is indeed LGI3, we sequenced the nucleotides of rat LGI3 PCR product. The result showed that rat LGI3 gene consisted of a predicted 1647 bases and exhibited 88.585% homology with the human LGI3 gene [Fig. 3(A)]. The first 60 bases of rat LGI3 and human LGI3 displayed low homology [Fig. 3(A)]. Rat LGI3 protein consisted of 548 predicted amino acids and exhibited 95.985% homology with human LGI3 protein [Fig. 3(B)]. Variability in amino acid sequence between rat LGI3 and human LGI3 were mainly found among the N-terminal amino acids [Fig. 3(B)], which was consistent with the

A

B

Fig. 3. (A) Alignment of deduced nucleotide sequence of rat LGI3 gene and homology comparison with human LGI3 gene. Rat and human LGI3 shared 88.585% homology. (B) Alignment of deduced amino acid sequence of rat LGI3 and homology comparison with human LGI3. Rat and human LGI3 amino acids shared 95.985% homology. (C) The schematic structure of deduced protein domains of rat LGI3 was constructed using software available at http://www.ensembl.org/. Signal peptide domain (1–30 amino acids); LRR domains (89–112, 113–136, 137–160, 170–219 amino acids); EPTP domains (223–263, 411–452 amino acids); transmembrane domain (288–310 amino acids). (D) The deduced structure of LGI3 protein was constructed by using MOE. The *yellow arrow* represents the β-sheet domain. The *red arrow* represents the α-helix domain.

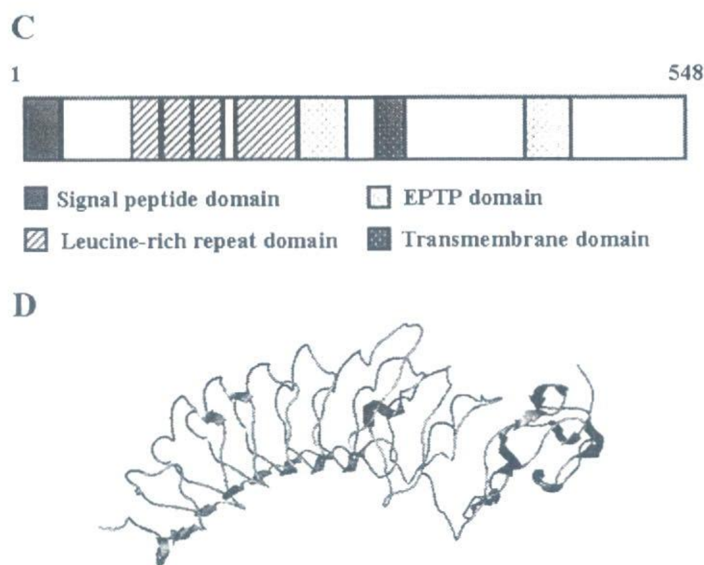

Fig. 3. Continued.

finding that the first 60 bases of the rat and human LGI3 genes shared little homology [Fig. 3(A)]. A schematic structure of the deduced protein domains of rat LGI3 is shown in Fig. 3(C), and a MOE-modeled structure of the deduced LGI3 protein is shown in Fig. 3(D).

Western Blot Analyses

Although Aβ treatment increased LGI3 expression, this did not necessarily indicate that Aβ also affected LGI3 protein expression. To determine whether Aβ influences LGI3 protein expression, we assessed LGI3 protein levels by Western Blotting using anti-TA142 antibody, the antibody we prepared against LGI3. We also examined astroglial responses to Aβ treatment with Western Blotting. In blots of samples prepared from rat astrocyte cultures, anti-TA142 antibody immunostained a ~60-kDa band representing LGI3 (Fig. 4). TA142 immunoreactivity was completely abolished by preabsorption with specific antigen peptide (data not shown).

After 3 h of Aβ treatment (5 μM Aβ40 or Aβ42), LGI3 protein expression increased significantly, with Aβ42 causing a greater increase than that caused by Aβ40 (Fig. 4). Within the same time frame, Aβ treatment also increased GFAP and ApoE expression (Fig. 4). This increase was significant in Aβ42-treated samples. In blots of samples prepared from rat primary cerebral cortical cultures, neither Aβ40 nor Aβ42 affected LGI3 protein expression (data not shown). After 24 h of Aβ treatment, LGI3, GFAP, and ApoE expression decreased to almost baseline levels [Fig. 4(B)].

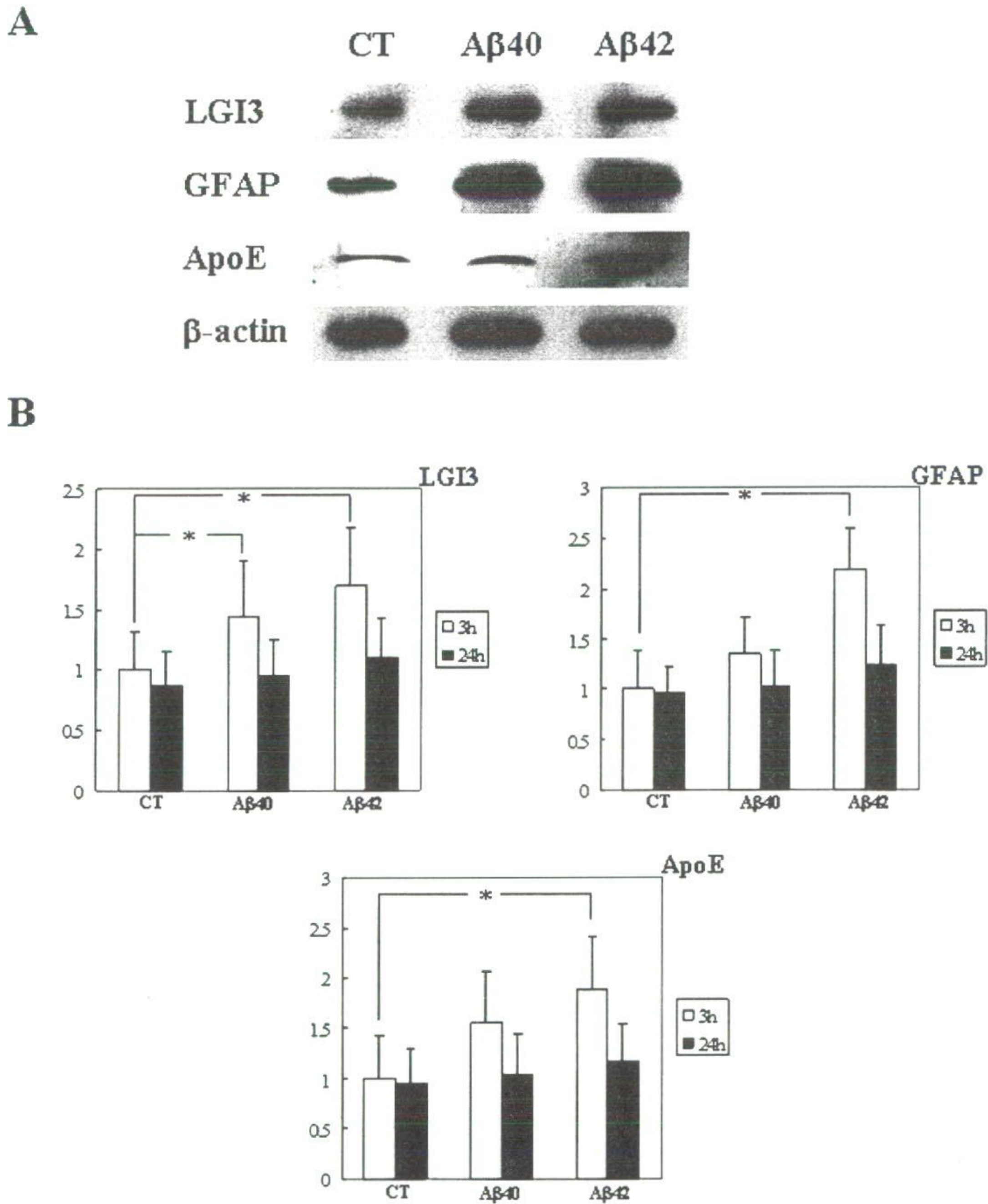

Fig. 4. (A) Western Blots showing the protein expression profiles of LGI3, GFAP, ApoE, and β-actin in extracts from rat astrocyte cultures following 3 h-treatment with Aβ. In this experiment, Aβ40 and Aβ42 were diluted in culture medium to a final concentration of 5 μM. (B) After 3 h of Aβ treatment using either Aβ40 or Aβ42, LGI3 protein levels in rat astrocyte cultures significantly increased. Both GFAP and ApoE also significantly increased with Aβ42 treatment within the same time frame. After 24 h of Aβ treatment, the increases in LGI3, GFAP, and ApoE similarly dropped. We did not find any significant change. All data were normalized according to β-actin protein levels (control). In this analysis, normalized data from control group after 3 h treatment were used as the standard for *Y* axis. Values are means ± SD. $^{*}p < 0.02$. CT—control rat astrocyte cultures contained the same concentration of DMSO found in the Aβ treatment solutions; Aβ40—Aβ40 treatment; Aβ42—Aβ42 treatment; 3 h—after 3 h of Aβ treatment; 24 h—after 24 h of Aβ treatment.

Immunocytochemistry

To confirm that LGI3 localizes within astrocytes, we immunostained rat astrocyte cultures with anti-TA142 antibody. Anti-TA142 immunostained the cytoplasm, plasma membranes, and nuclei of cultured astrocytes [Fig. 5(A–D)]. To investigate the relationship between LGI3 and Aβ, we double immunostained Aβ-treated cultured astrocytes with antibodies against LGI3 and Aβ. In control cultures without Aβ treatment [Fig. 5(E–H)], no immunostaining was observed with anti-Aβ antibody, confirming that this antibody, 4G8, raised against human Aβ does not recognize endogenous rodent Aβ. In contrast, after 3 h of Aβ42 treatment, LGI3 and Aβ colocalized at plasma membranes [Fig. 5(I–L)]. At plasma membranes, both Aβ and LG13 immunostaining were punctate and granular in appearance [Fig. 5(I–L)]. In addition, we found that internalized Aβ also colocalized with LGI3 in the cultured astrocytes [Fig. 5(M–P)]. Both LG13 and internalized Aβ appeared as large granular matter [Fig. 5(M–P)].

DISCUSSION

A recent study showed that Aβ induces the expression of a transmembrane protein that contains LRRs in rat astrocytes (Satoh *et al.*, 2002). In the present study, we focused on the LGI family, which also encodes a type I transmembrane protein containing LRRs (Gu *et al.*, 2002). We used molecular, biological, and biochemical techniques to assess how Aβ influences the LGI family.

We found that Aβ induced LGI3 expression in astrocytes after 3 h of Aβ treatment (Fig. 1). This is the first study to show that astrocytes respond to Aβ by increasing the expression of LG13, a LGI family member. Since we did not observe a significant increase in LGI3 expression after 24 h of Aβ treatment, we conclude that the Aβ-induced upregulation of LGI3 may reflect an early-stage astroglial response against Aβ.

To determine the expression pattern of LGI3 gene across different brain regions and different types of neural cells, we assessed LGI3 expression in rat brain and in primary cerebral cortical cultures and astrocyte cultures (Fig. 2). In rat brain, the expression of LGI3 gene was highest in cerebral cortex [Fig. 2(A)], indicating that LGI3 may be mainly expressed in cortical neural cells. This is consistent with the finding that human LGI3 is mainly expressed in the brain, and that human LGI1 protein, which is highly homologous to rat LGI3 protein, is also strongly expressed in human brain (Gu *et al.*, 2002). Quantitatively, however, LGI3 was expressed more abundantly in cultured astrocytes than in cultured neurons [Fig. 2(B)]. This finding indicated that LGI3 may be more strongly associated with astroglial functions rather than with neuronal functions; thus, it would be logical to investigate LGI3 function in cultured astrocytes.

A comparison of rodent and human LG13 nucleotide and amino acid sequences revealed that LGI3 is highly conserved, thereby supporting our use of rat cultured astrocytes to investigate the relationship between LGI3 and Aβ. From the modeled structure of the deduced protein domains of LGI3, we predicted that the LRR

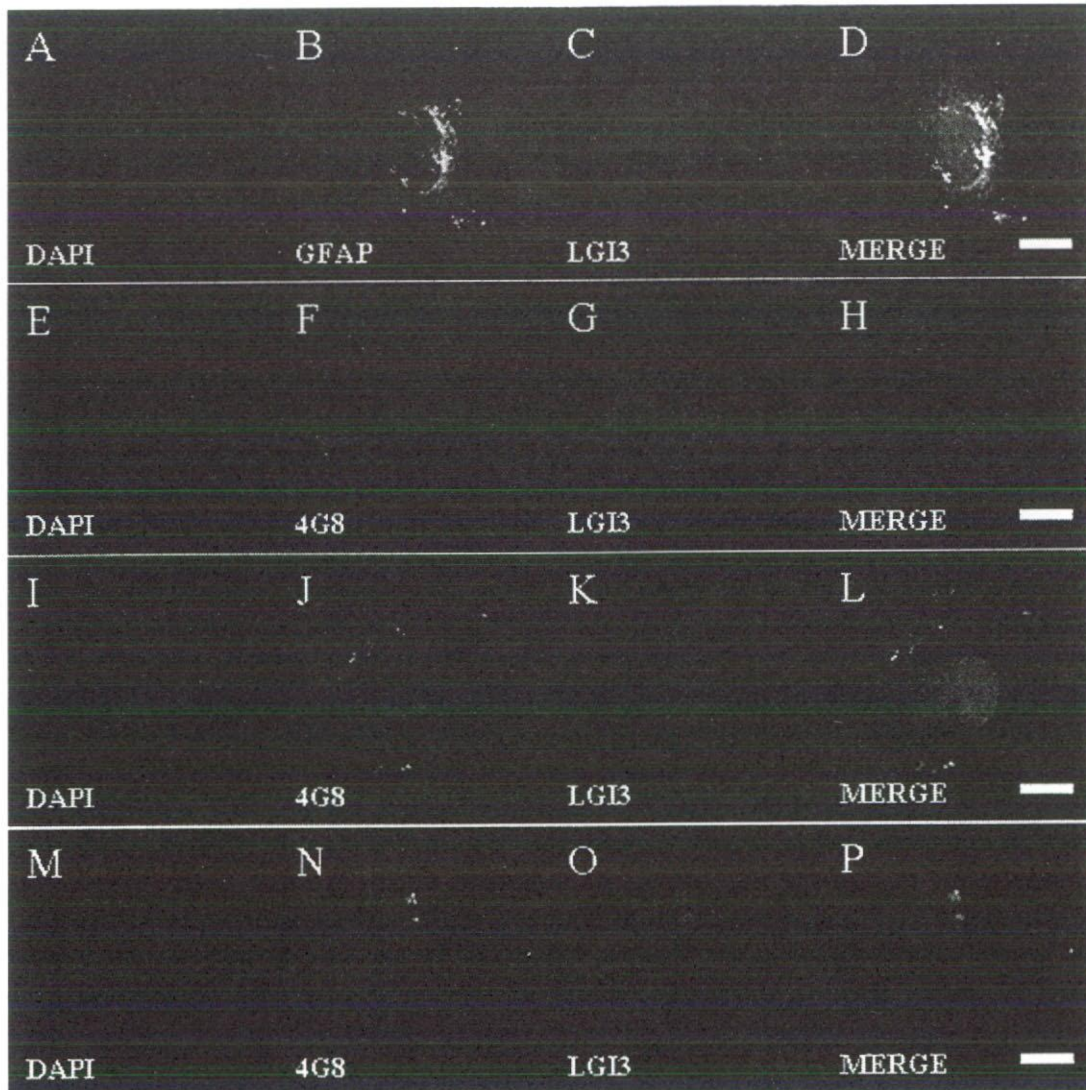

Fig. 5. (A–D) Photomicrographs of cultured rat astrocytes. Cells were immunostained with anti-TA142 antibody and anti-GFAP antibody 6F2, then counterstained with the nuclear marker DAPI. Anti-TA142 immunostained the cytoplasm, plasma membranes, and nuclei of cultured rat astrocytes (A–D). (E–H) Photomicrographs of cultured rat astrocytes immunostained with anti-TA142 antibody and anti-Aβ antibody 4G8, followed by DAPI. In these untreated control cultures, 4G8 did not immunostain endogenous Aβ. (I–P) Photomicrographs of Aβ-treated astrocytes immunostained with anti-TA142 antibody and anti-Aβ antibody 4G8, followed by DAPI. After 3 h of Aβ treatment, 4G8-immunoreactive Aβ localized at the plasma membranes of some cultured astrocytes; TA142-immunoreactive matter representing LGI3 similarly localized to plasma membranes (I–L). We also found that LGI3 colocalized with large, granular Aβ deposits that had been internalized by some astrocytes (M–P). Scale *bars*: 10 μm.

domains were outside the transmembrane domain [Fig. 3(D)], indicating that interactions between LGI3 and Aβ should occur extracellularly.

To confirm the Aβ-induced pattern of LGI3 gene expression (Fig. 1), we assessed LGI3 protein expression in rat primary cerebral cortical cultures and astrocyte cultures treated with Aβ. Western Blot analyses showed that Aβ also influences

LGI3 protein expression in astrocyte cultures (Fig. 4), clearly supporting our initial findings that LGI3 expression was induced by Aβ (Fig. 1). Many studies have shown that astrocytes are affected by soluble neuronal factors and several inflammation-associated cytokines (Eddleston and Mucke, 1993; Mark *et al.*, 1995; McGeer and McGeer, 1995) and by Aβ (LaDu *et al.*, 2001; Smits *et al.*, 2002; Deb *et al.*, 2003). Aβ40, the Aβ species thought to be the primary form of Aβ found in normal brain (Haas *et al.*, 1992; Seubert *et al.*, 1992; Shoji *et al.*, 1992; Busciglio *et al.*, 1993; Vigo-Pelfrey *et al.*, 1993), is less toxic than Aβ42 (Burdick *et al.*, 1992; Jarrett *et al.*, 1993; Suzuki *et al.*, 1994; Younkin, 1994).

In the present study, Aβ40 induced LGI3 expression in astrocytes to nearly the same extent as did Aβ42 (Figs. 1 and 4). Since astrocytes primarily have a supportive role in the brain, these results suggest that LGI3 may be mostly associated with not only Aβ toxicity but also with other biological functions. Several studies have also shown that Aβ activates astrocytes, which then take up Aβ for degradation (Funato *et al.*, 1998; Matsunaga *et al.*, 2003; Wyss-Coray *et al.*, 2003), and that Aβ induces astrocytes to produce ApoE and chemokines (LaDu *et al.*, 2001; Smits *et al.*, 2002; Deb *et al.*, 2003). In the present study, Aβ42-induced increase in GFAP and ApoE protein levels in astrocytes occurred coincidentally with that of LGI3 (Fig. 4). These increases also similarly decreased after 24 h of Aβ treatment (Fig. 4). Taken together, these findings suggest that LGI3 may be involved in the GFAP- and ApoE-related astroglial responses against Aβ.

Immunocytochemical analyses revealed that LGI3 localized not only within the cytoplasm but also at the plasma membranes of astrocytes [Fig. 5(A–D)]. Since LGI3 encodes a transmembrane protein that contains LRRs (Fig. 3), which were demonstrated by amino acid sequence analyses to be located within the putative extracellular part of LGI3 [Fig. 3(B)], it is conceivable that LGI3 would most likely exist as a transmembrane protein in astrocytes. However, we found that LGI3 also localized within the nuclei of astrocytes [Fig. 5(A–D)]. Several studies have shown that with some transmembrane molecules, such as Notch and amyloid precursor protein, ligand binding or a specific secretase can induce the cleavage and release of the intracellular domain, which then translocates to the nucleus and functions as a transcription factor (Greenwald, 1998; Sastre *et al.*, 2001; Yu *et al.*, 2001; Chen *et al.*, 2002; Ebino and Yankner, 2002; Weidemann *et al.*, 2002). Since anti-TA142 antibody recognizes the intracellular domain of LGI3, the TA142 immunoreactivity we observed in nuclei may represent cleaved LG13. Anti-TA142, however, immunostained a ~60-kDa band representing full-length LGI3 in our Western Blot analyses (Fig. 4). Thus, it still remains to be determined whether LGI3 is cleaved or whether LGI3 has different functions in different subcellular compartments.

Double immunostaining analyses showed that LGI3 at plasma membranes colocalized with Aβ [Fig. 5(I–L)] and that internalized Aβ colocalized with LGI3 in astrocytes [Fig. 5(M–P)]. Since LRR proteins are thought to be involved in protein–protein interactions (Kobe and Deisenfofer, 1994; Buchanan and Gay, 1996), Aβ may induce and then even bind LGI3 such that Aβ-bound LGI3 may play a role in Aβ-related signal transduction cascade(s) or in Aβ endocytosis in astrocytes.

In summary, we identified a novel Aβ-associated protein, LGI3, that may be involved in astroglial responses against Aβ. Additional research is needed to determine the precise function of LGI3 in brain and to clarify its relationship with Aβ *in vivo*. Data from such studies would contribute greatly to clarifying the role of astrocytes in brain.

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Discrimination of Antibody to Herpes B Virus from Antibody to Herpes Simplex Virus Types 1 and 2 in Human and Macaque Sera▽

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The antigenic cross-reactive characteristics of herpes B virus and herpes simplex virus (HSV) type 1 (HSV-1) and HSV-2 are responsible for false-positive diagnoses by serological assays in humans and macaques. In the present study, we developed a fluorometric indirect enzyme-linked immunosorbent assay (ELISA) with recombinant herpes B virus glycoprotein D (gD) and HSV-1 and HSV-2 gG (gG-1 and gG-2, respectively) to discriminate between the three primate herpesvirus infections. The secreted form of gD, gDdTM, was used to detect antibody to herpes B virus gD. Sera positive for herpes B virus, HSV-1, and HSV-2 showed specific reactions to gD, gG-1, and gG-2, respectively. Sera collected from humans and rhesus macaques were investigated for the presence of antibodies to the recombinant proteins of the three herpesviruses. The results suggested that the approach is able to discriminate between herpes B virus and HSV infections. The ELISA was also found to be able to detect infections with multiple primate herpesviruses and may have the potential to identify a subsequent infection in individuals that have already been infected with another herpesvirus. In addition, we found evidence of a greater cross-reactivity of herpes B virus with HSV-1 than with HSV-2. It is suggested that the ELISA with the recombinant antigens is useful not only for the serodiagnosis of primate herpesvirus infections but also for elucidation of the seroprevalence of herpesviruses in humans and primates.

Herpes B virus (*Cercopithecine herpesvirus* 1) infection is a fatal zoonosis characterized by acute encephalomyelitis (26, 27). The rate of mortality among individuals with the infection is high if such individuals are not given antiviral therapy in the early stages of infection. The natural hosts of the causative agent are Asian macaques, which are used in the medical field as models for humans. This suggests that laboratory workers in contact with the macaques could become exposed to virus-contaminated sources, such as saliva and urine from infected hosts (4). Therefore, the development of a rapid and accurate method for the detection of herpes B virus infection is required for both the early diagnosis of the infection in patients and the establishment of virus-free macaque colonies. Serological assays, including enzyme-linked immunosorbent assay (ELISA) and Western blotting (WB) analysis with a herpes B virus-infected cell antigen, are available for the detection of herpes B virus infections (2, 7, 12, 18).

The serodiagnosis of herpes B virus infections is difficult because of the antigenic cross-reactivity of herpes B virus with related herpesviruses. Herpes B virus is classified as a member of the subfamily *Alphaherpesvirinae*, which includes herpes simplex virus (HSV) type 1 (HSV-1) and HSV-2, and has been shown to share antigenic and biological characteristics with these human herpesviruses, such as a tropism for neurons and propagation and dissemination in natural hosts (6, 8, 21). The high seroprevalence of HSV in humans, which has been reported to be 60 to 88% for HSV-1 (3, 5, 28, 29), limits the detection of herpes B virus infection by serological tests in patients suspected of being infected with the virus. In addition, a biosafety level 4 laboratory is required for preparation of the virus-infected cell antigen. Therefore, an alternative antigen as a replacement for the infected cell antigen is needed for the serological diagnosis of herpes B virus infections.

Recombinant DNA techniques currently play an important role in the diagnosis of many viral infections. The recombinant proteins used as antigens in serological tests are particularly useful for the discrimination of antibodies to closely related viruses. Immunoassays with glycoprotein G (gG) of HSV-1 and HSV-2 (gG-1 and gG-2, respectively), which are known to be type-specific antigens (16, 25), have been developed for the typing of HSV (1, 9, 14, 15, 22) and are available commercially. These assays have been applied in epidemiological studies as well as to the serological diagnosis of infections in patients. In addition, the development of serological assays for the diagnosis of herpes B virus infection with the recombinant protein has been reported (20, 24). In an earlier study we produced the

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gD of herpes B virus in mammalian cells; and the resultant recombinant protein was evaluated for its antigenicity by WB, dot blotting, and immunoprecipitation analyses (24). Since a nonspecific reaction was observed by WB, we constructed the secretory form of gD, gDdTM, which lacked the transmembrane domain (TM) and cytoplasmic tail (CT). gDdTM showed a specific reaction with sera from herpes B virus-infected macaques and was confirmed to have the same sensitivity as the original gD antigen. Therefore, we concluded that the gDdTM antigen is useful for the detection of antibody to herpes B virus.

In the present study, we developed a fluorometric indirect ELISA with a combination of recombinant herpes B virus gD, gG-1, and gG-2 as coating antigens. We used the gDdTM described above to detect antibody to herpes B virus. The three antigens were investigated for their cross-reactivities with sera confirmed to have antibody to herpes B virus or HSV. Sera from rhesus macaques and humans, including patients with meningitis or myelitis, were also examined for the presence of antibody to herpes B virus, HSV-1, or HSV-2. The results were used to evaluate the ability of the ELISA to discriminate between the three herpesvirus infections.

MATERIALS AND METHODS

Antigens. The preparation of recombinant herpes B virus gDdTM has been described previously (24). A recombinant plasmid, pBgDdTM, was used to transfect COS7 cells. The supernatant containing the resultant gDdTM was used as a coating antigen, while the supernatant of the COS7 cells transfected with an empty vector, pcDNA3.1(−), was used as the negative coating antigen. The recombinant gG-1 and gG-2 antigens and the whole HSV-1 and HSV-2 antigens were purchased from Austral Biologicals and Biogenesis Ltd. (Poole, United Kingdom), respectively.

Serum samples. Polyclonal antisera with antibodies to HSV-1 and HSV-2 were collected from rabbits experimentally immunized with HSV-1 and HSV-2 (11). The complement fixation titers to HSV for the anti-HSV-1 and anti-HSV-2 rabbit sera were 1:256 and 1:128, respectively (10). Human control sera confirmed to have HSV-1 or HSV-2 antibody were also used for evaluation of the ELISA developed in the present study. The control serum sample for HSV-1 was obtained from a person with no clinical symptoms. This serum sample was confirmed to have a complement fixation titer to HSV of 128 (10) and neutralizing antibody titers to HSV-1 and HSV-2 of 64 and 4, respectively (13). Serum obtained from a patient with meningitis was used as the control for HSV-2 (17). Antibody to gG-2 was qualitatively detected in this patient's serum by a type-specific ELISA, and amplified products of HSV were obtained from the cerebrospinal fluid of this patient, although the virus type was not determined. Control serum with antibody to herpes B virus was obtained from a rhesus macaque that was naturally infected with the virus. The antibody to herpes B virus in this serum was qualitatively detected by ELISA with inactivated herpes B virus antigen (23). In addition, 24 and 21 serum samples were collected from rhesus macaques and persons with no clinical symptoms, respectively. Five convalescent-phase serum samples were obtained from patients diagnosed with central nervous system HSV infections (17).

Fluorometric indirect ELISA. Ninety-six-well microplates (Maxisorp immunoplate; Nalge Nunc, Tokyo, Japan) were coated with the recombinant or HSV antigens diluted in carbonate buffer overnight at 4°C. The supernatants of COS7 cells transfected with pBgDdTM or pcDNA3.1(−) were diluted 1:500 and were used as the coating antigen. Ten nanograms per well of the gG-1 or gG-2 antigen was used for the recombinant antigen-based ELISA, whereas 100 ng per well of HSV-1 or HSV-2 antigens was used for the whole-virus antigen-based ELISA. The prepared plates were blocked with blocking buffer (phosphate-buffered saline [PBS] containing 3% bovine serum albumin) for 2 h at room temperature. After each incubation step, the plates were washed three times with PBS containing 1% Tween 20 (PBST) and four times before the enzyme-substrate reaction step. The serum samples were serially diluted fourfold from 1:100 to 1:25,600 with dilution buffer (PBST containing 1% bovine serum albumin). One hundred microliters of the diluted serum was added to each well, and the plate was incubated for 2 h on a plate shaker at room temperature. Biotin-conjugated

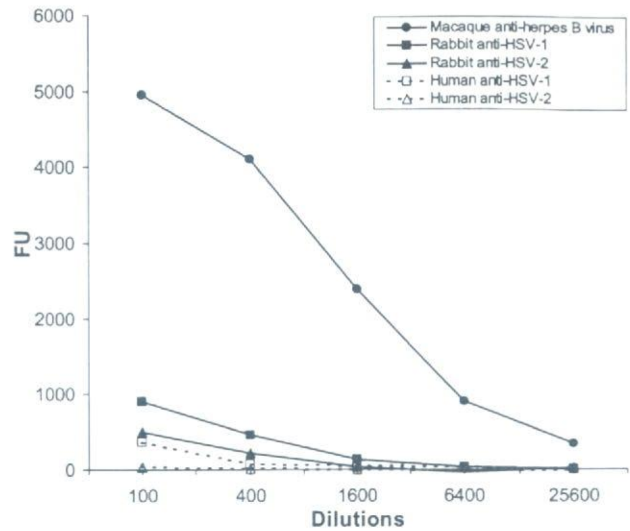

FIG. 1. Reactivity of the control sera with the herpes B virus gDdTM antigen. The results obtained with rhesus macaque serum which contained antibody to herpes B virus, rabbit sera immunized with HSV-1 or HSV-2, and human serum which contained antibody to HSV-1 or HSV-2 are shown. Sera were serially diluted fourfold from 1:100 to 1:25,600, and the FU values for each dilution were obtained by the fluorometric indirect ELISA with herpes B virus gDdTM. The FU values were plotted against each dilution of serum. The resulting titration curves are shown.

secondary antibodies were used in the present study. Donkey anti-rabbit immunoglobulin G (IgG; Chemicon International Inc.) diluted 1:100,000 in the dilution buffer, goat anti-monkey IgG γ chain (Rockland Immunochemicals Inc.) diluted to a concentration of 25 ng/ml, and goat anti-human IgG (Fc) (American Qualex International Inc., CA) diluted to a concentration of 6.25 ng/ml were used for the detection of rabbit, monkey, and human IgG, respectively. The secondary antibody reaction step was performed for 1 h on the plate shaker at room temperature. Streptavidin-conjugated β-galactosidase was diluted at 1:1,000 in the dilution buffer, and 100 μl was added to each well. The reaction was performed for 1 h on the plate shaker at room temperature. The enzyme-substrate reaction with a 0.2 mM 4-methylumbelliferyl-β-D-galactoside substrate solution was performed for 2 h at 37°C and was stopped by adding 0.1 M glycine (pH 10.3). The amount of fluorescent reactant was calculated as the number of fluorescence units (FUs) after measurement of the absorbance at 460 nm with a fluorometric microplate reader (Fluoroskan II; Labsystems, Tokyo, Japan). The FU values for the positive antigens subtracted from those for the negative antigens were used to evaluate the reaction in the ELISA. Reactions with values of less than 500 were considered negative. Antibody titers were taken as the reciprocal of the final dilutions on titration curves which gave positive reactions.

RESULTS

Antigenic specificity of recombinant herpes B virus gD. The reactivities of control sera for herpes B virus, HSV-1, or HSV-2 against the secretory form of herpes B virus gD lacking TM and CT (gDdTM) were investigated. Serum collected from rhesus macaques naturally infected with herpes B virus showed high levels of reactivity to the recombinant antigen (Fig. 1). The FU values for serial dilutions of the serum samples were almost linear, suggesting that specific binding between the coated antigen and the antibody in the serum occurred. The titer was 6,400. Sera from an uninfected macaque, rabbit, and human had low FU values (less than 500), suggesting no reactivity to the positive antigen (data not shown). Although the serum sample from a rabbit immunized with HSV-1 showed a

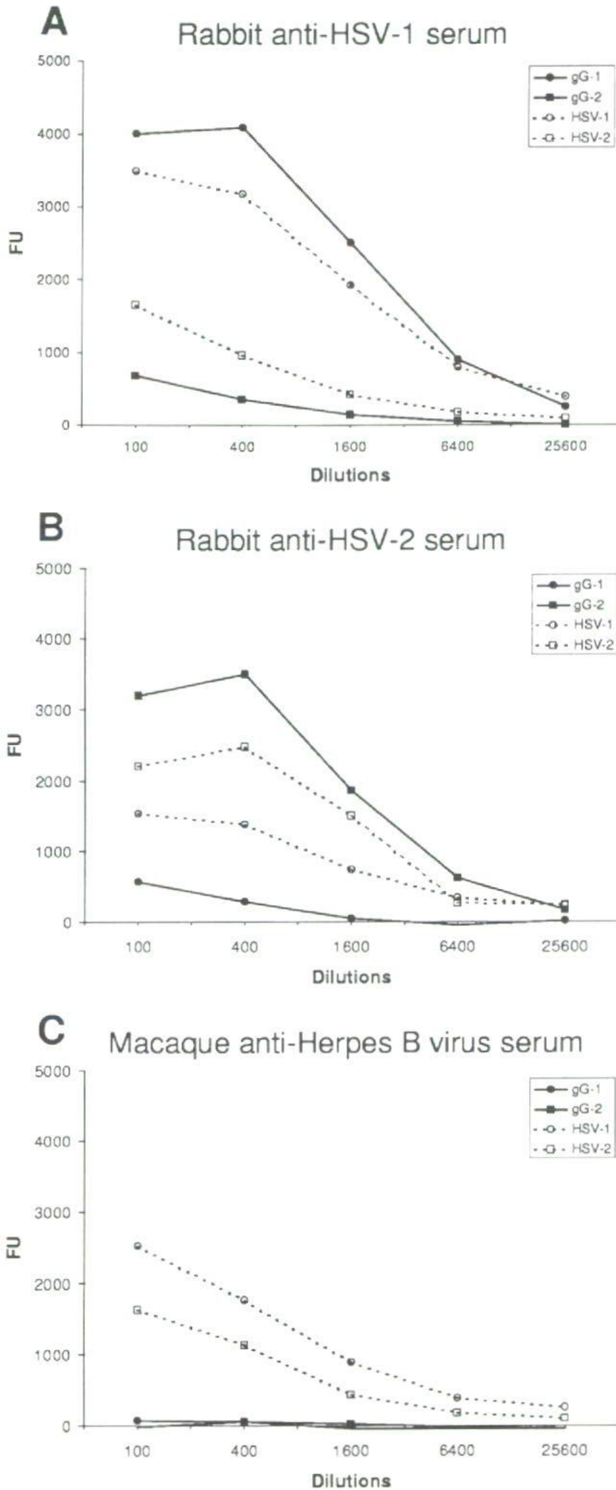

FIG. 2. Reactivity of the control sera with HSV gG-1 or gG-2 and whole-virus (HSV-1 or HSV-2) antigens. The results for rabbit anti-HSV-1 sera (A), rabbit anti-HSV-2 sera (B), and rhesus macaque anti-herpes B virus sera (C) are shown. The titration curves were obtained as described in the legend to Fig. 1, except that gG or HSV was used as the coating antigen. In each panel, four titration curves show the reactivity of the serum with the gG-1, gG-2, HSV-1, and HSV-2 antigens.

TABLE 1. Titers of antibodies to recombinant or whole primate herpesvirus antigens in rhesus macaque sera

Monkey no.	Antibody titer[a]				
	Anti-gD	Anti-gG-1	Anti-gG-2	Anti-HSV-1	Anti-HSV-2
7	UD	UD	UD	UD	UD
8	UD	UD	UD	UD	UD
9	UD	UD	UD	UD	UD
10	UD	UD	UD	UD	UD
11	UD	UD	UD	UD	UD
1308	200	UD	UD	200	UD
1309	UD	UD	UD	UD	UD
1333	3,200	UD	UD	800	UD
1371	3,200	UD	UD	UD	UD
1373	800	UD	UD	800	UD
1376	UD	UD	UD	UD	UD
1379	3,200	UD	UD	400	UD
1381	ND[a]	ND	ND	ND	ND
1383	UD	UD	UD	UD	UD
1385	UD	UD	UD	UD	UD
1386	3,200	UD	UD	3,200	800
1395	UD	UD	UD	UD	UD
1401	6,400	1,600	UD	3,200	800
1402	1,600	UD	UD	1,600	400
1403	1,600	UD	UD	1,600	UD
1404	UD	UD	UD	UD	UD
1413	3,200	UD	UD	3,200	200
1416	1,600	UD	UD	1,600	400
1417	800	UD	UD	800	UD

[a] Abbreviations: UD, under the detection limit (titer, <100); ND, not determined.

slight cross-reaction with a titer of 100 (Fig. 1), the other HSV-1- or HSV-2-infected rabbit and human serum samples showed no reactivity to the antigen of herpes B virus.

Antigenic specificity of recombinant gG-1 and gG-2. Anti-herpes B virus macaque serum and anti-HSV-1 and anti-HSV-2 rabbit and human sera were investigated for their reactivities to gG-1 or gG-2 by the fluorometric indirect ELISA. The results were compared with those obtained by the ELISA with the HSV-1 or the HSV-2 antigen. Virus-uninfected macaque, rabbit, and human sera did not react with any recombinant or whole-virus antigen (data not shown). Sera from the rabbits infected with HSV-1 or HSV-2 reacted not only with the homologous antigens but also with the heterologous antigens in the gG- and HSV-based ELISAs (Fig. 2A and B). The antibody titers obtained under the homologous antigen-antibody conditions, however, were higher than those obtained under the heterologous antigen-antibody conditions. Under the homologous conditions, the titers obtained by the gG-based ELISA were higher than those obtained by the HSV-based ELISA, while under the heterologous conditions, the titers showing the reaction to gG were lower than those showing the reactions to the virus antigens. In addition, the human control serum for HSV-1 reacted only with the homologous antigens, and the reactivity to gG-1 was higher than that to the HSV-1 antigen (data not shown). The human control serum for HSV-2 showed a notably higher reaction to gG-2 than to any of the other antigens tested (data not shown). A macaque anti-herpes B virus serum was found to cross-react with the whole-virus antigens but not with gG (Fig. 2C).

Application to rhesus macaque sera. Twenty-four serum samples from rhesus macaques in a laboratory facility were

TABLE 2. Titers of antibodies to herpes B virus, HSV-1, and HSV-2 in humans with no clinical symptoms

Control subject no.	Antibody titer				
	Anti-gD	Anti-gG-1	Anti-gG-2	Anti-HSV-1	Anti-HSV-2
1	UD[a]	3,200	UD	3,200	UD
2	UD	6,400	UD	3,200	200
3	UD	UD	UD	UD	UD
4	UD	UD	UD	UD	UD
5	UD	UD	UD	UD	UD
6	UD	800	800	400	UD
7	UD	3,200	UD	3,200	200
8	UD	3,200	UD	3,200	200
9	UD	3,200	UD	3,200	UD
10	UD	UD	UD	UD	UD
11	UD	UD	UD	UD	UD
12	UD	6,400	UD	3,200	400
13	UD	UD	UD	UD	UD
14	UD	UD	UD	UD	UD
15	UD	UD	UD	UD	UD
16	UD	UD	UD	UD	UD
17	UD	UD	UD	UD	UD
18	UD	UD	UD	UD	UD
19	UD	UD	UD	UD	UD
20	UD	6,400	UD	6,400	400
21	UD	3,200	UD	6,400	400

[a] UD, under the detection limit (<100).

examined for the presence of antibodies to the five antigens gDdTM, gG-1, gG-2, HSV-1, and HSV-2. The antibody titers were calculated for each antigen (Table 1). Twelve of the 24 macaque serum samples were found to have antibody to the herpes B virus gD. Among these 12 serum samples, 1 showed reactivity to the gG-1 antigen and none showed reactivity to the gG-2 antigens, whereas 11 had antibodies to HSV-1 and 5 had antibodies to HSV-2. The titer of antibody to HSV-1 was higher than that to HSV-2 in five serum samples (serum samples 1386, 1401, 1402, 1413, and 1416) in which antibodies to both HSV-1 and HSV-2 were detected.

Assessment of human control and patient sera. Twenty-one serum samples collected from human controls were investigated for antibodies to the five antigens (Table 2). None of the serum samples had antibody to gDdTM. Of the 21 serum samples, 9 were found to have antibody to gG-1 and 1 was found to have antibody to gG-2. The results obtained by the ELISA with gG-1 were identical to those obtained by the ELISA with HSV-1, whereas the results of the ELISAs with gG-2 and HSV-2 were not identical.

Five serum samples from patients diagnosed with central nervous system HSV infections were examined for the presence of antibodies to the five antigens (Table 3). None of the serum samples had antibody to gDdTM. The results obtained by the gG-based ELISA developed in the present study were the same as those obtained by the previous ELISA (17): two serum samples (serum samples P-1 and P-4) had antibodies to both gG-1 and gG-2, two (serum samples P-2 and P-3) had only anti-HSV-2 antibody, and the fifth (serum sample P-5) did not have antibody to either gG-1 or gG-2. However, three serum samples (serum samples P-1, P-2, and P-4) showed reactivity to both HSV antigens, whereas the other two serum samples did not react with any viral antigens.

DISCUSSION

Sensitive reactions to recombinant antigen gDdTM, gG-1, and gG-2 were shown by the control sera from rhesus macaques, rabbits, and humans. Although a slight cross-reaction was observed, the ELISA with the recombinant antigens was confirmed to show specificity for the detection of herpes B virus and HSV infections. The specificity of herpes B virus gD was also demonstrated, and it is proposed that gD may be a valuable diagnostic reagent for the identification of herpes B virus infections (19, 20). In our study, all macaque sera except the serum from one individual that reacted to the HSV antigen were also confirmed to have antibody to gDdTM but not to gG. In contrast, all human control and patient serum samples positive for the whole-virus antigen had antibody to gG-1 and/or gG-2 but did not have antibody to gDdTM. The limited detection of antibody to the recombinant proteins in the only natural hosts supports the specificity of the recombinant antigen-based ELISA. Taken together, we suggest that use of the combination of the recombinant proteins from herpes B virus and HSV is suitable for the discrimination of herpes B virus infection from HSV infection.

The herpes B virus recombinant antigen, gDdTM, does not contain the TM and CT regions, in which a linear B-cell epitope spanning residues 362 to 370 was found (19). In our previous study, we found that some serum samples seropositive for herpes B virus failed to react to gDdTM by WB analysis but could be found to have antibody to this secretory form of the protein by dot blot analysis (24). In accordance with our findings, the recombinant gD lacking the linear epitope was found to have a reduced reactivity to anti-herpes B virus serum under denatured conditions, suggesting the presence of conforma-

TABLE 3. Titers of antibodies to recombinant or whole primate herpesvirus antigens in patient sera

Patient no.	Diagnosis	Virus(es) detected by[a]:		Antibody titer				
		PCR	ELISA	Anti-gD	Anti-gG-1	Anti-gG-2	Anti-HSV-1	Anti-HSV-2
P-1	Myelitis	HSV-2	HSV-1 and HSV-2	UD[b]	3,200	3,200	200	200
P-2	Meningitis	HSV[c]	HSV-2	UD	UD	800	400	400
P-3	Meningitis	ND[d]	HSV-2	UD	UD	800	UD	UD
P-4	Meningitis	ND	HSV-1 and HSV-2	UD	800	400	3,200	200
P-5	Meningitis	HSV-1	ND	UD	UD	UD	UD	UD

[a] DNA and detection of antibodies to HSV-1 and HSV-2 were performed by PCR and a gG-based ELISA, respectively, in a previous study (19).
[b] UD, under the detection limit (<100).
[c] DNA was amplified from the cerebrospinal fluid of patient P-2, but the type could not be determined (17).
[d] ND, not detected.

tion-dependent epitopes in the extracellular domain (19). In the present study, gDdTM was used under nondenatured ELISA conditions. The results showed that an anti-herpes B virus macaque serum reacted strongly with the secreted form of herpes B virus gD, whereas a negative serum did not. In addition, the investigation of rhesus macaque sera showed that the gD-based ELISA did not fail to detect antibody in any serum sample which cross-reacted with the HSV antigens. Thus, it is suggested that the ELISA developed is able to detect antibodies by recognizing the epitopes in the extracellular domain.

Although we did not compare the sensitivity and specificity of the recombinant gD antigen with those of the whole herpes B virus antigens, the gG antigen was evaluated by comparison with the HSV antigen in experiments with anti-HSV-1 and anti-HSV-2 sera. The titers obtained by the gG-based ELISA were higher than those obtained by the HSV-based ELISA under the homogeneous antigen-antibody conditions, suggesting that the sensitivities of the recombinant proteins were higher than those of the whole-virus antigens. In contrast to this finding, the titers obtained by the recombinant antigen-based ELISA were lower than those obtained by the whole-virus-antigen-based ELISA under the cross-reactive conditions between HSV-1 and HSV-2, suggesting that the specificity for the recombinant antigens was greater than that for the whole-virus antigens.

A sample of macaque serum (serum sample 1401) was found to have antibody not only to gG-1 but also to herpes B virus gD. The other macaque serum sample with herpes B virus infection, however, did not have antibody to either gG-1 or gG-2. These results suggest a specific reaction of the serum sample (serum sample 1401) to gG-1. We concluded that this macaque had multiple virus infections (i.e., it was infected with HSV-1 as well as herpes B virus), although we could not determine which virus affected this individual first. The macaques investigated had opportunities to be exposed to HSV-1 and HSV-2 from laboratory workers. However, no animals had antibody to gG-2. Macaques in laboratory facilities might have more frequent opportunities to be exposed to HSV-1 than to HSV-2 because of the higher prevalence of HSV-1 infection in humans (3, 5, 28, 29).

We examined the existence of antibodies to the recombinant herpes B virus or HSV proteins in sera from patients diagnosed with HSV meningitis or myelitis, since the clinical symptoms caused by HSV infections are almost the same as those caused by herpes B virus infection. We did not detect antibody to gDdTM in these samples, whereas most of the patients were found to have antibody to gG-1 and/or gG-2. Although we could not examine serum from patients with herpes B virus infections, the identification of multiple infections in a macaque serum sample suggests that the ELISA developed can detect antibody to herpes B virus even in patients who have already been infected with HSV. On the other hand, serum taken from one patient (patient P-5) was not found to contain antibody to gG-1 or gG-2, even though HSV-1 DNA was amplified from the patient's cerebrospinal fluid. No antibody to gG was detected in the serum of this patient in the previous study either (17). In addition, this serum sample was also found not to have antibody to either HSV-1 or HSV-2. Therefore, it appears that this patient did not produce IgG antibody in the serum. Further investigation, such as tests for the detection of IgM antibody, would be required.

Eleven of 12 macaque serum samples confirmed to have antibody to herpes B virus were found to show cross-reactivity with HSV-1, whereas only 5 showed cross-reactivity with HSV-2. In addition, in all HSV-1- and HSV-2-seropositive macaques, the titers of antibodies to HSV-1 were higher than those to HSV-2. These results suggest that herpes B virus has more antigenic cross-reactivity with HSV-1 than with HSV-2. This suggestion could be supported by the findings in a report by Eberle et al. (6), in which the cross-neutralization titers of anti-herpes B virus serum to HSV-1 were shown to be higher than those to HSV-2. Complete genomic sequence analysis of herpes B virus showed that there are 20 proteins which are more similar to HSV-1 proteins, including capsid proteins, whereas another 46 proteins are more similar to HSV-2 proteins and include DNA cleavage and packaging proteins (21). Therefore, the higher degrees of similarity of the structural proteins recognized by the humoral immune system might explain the higher cross-reactivity of herpes B virus with HSV-1 than with HSV-2. However, gD and gG are not likely to contribute to the cross-reaction between herpes B virus and HSV.

In summary, the fluorometric indirect ELISA with recombinant herpes B virus gD and HSV gG was shown to have the potential to discriminate between herpes B virus infection and HSV-1 and HSV-2 infections in humans and macaques. In addition to the clinical aspect, this ELISA would contribute to the assessment of the seroprevalence of alphaherpesvirus infections in humans and primates, including the natural hosts of herpes B virus.

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