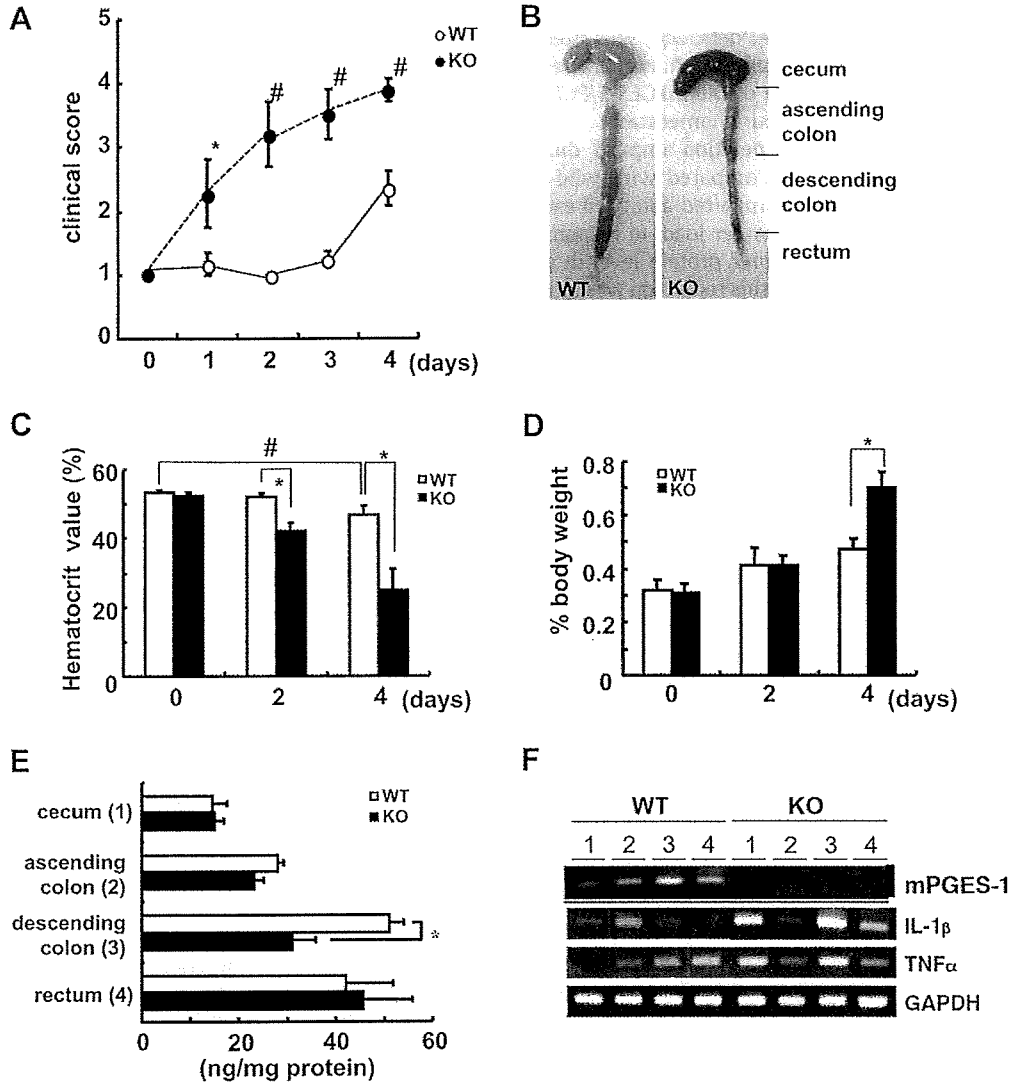

Fig. 3. Exacerbation of DSS-induced colitis in mPGES-1 knockout mice. DSS of the average molecular weight of 5000, at 5% concentration in the drinking water, was administered orally into mPGES-1 wild-type or knockout mice for 4 days. A. The fecal blood score was monitored daily. Fecal blood was scored as follows: 1, normal; 2, trace positive; 3, positive; 4, strong positive; 5, gross bleeding. B. The macroscopic examination of the large intestines on day 4 is shown. Hemorrhagic redness throughout the cecum to the rectum in the large intestine of mPGES-1 knockout mice. C and D. On days 0, 2, and 4, hematocrit values and spleen weights were measured. The weights of spleen are shown as % body weight. E and F. On day 4, the large intestines were divided into four regions. PGE_2 levels and mPGES-1, IL-1β, TNFα mRNA levels in each region (cecum (lane 1), ascending colon (lane 2), descending colon (lane 3), and rectum (lane 4)) were analyzed by EIA and RT-PCR, respectively. Data were analyzed by Student's *t*-test. The results are expressed as means ± standard error ($n = 8–12$; *, $p < 0.05$ versus wild-type mice).

cases, some exceptions have been reported. Treatment of rats with LPS resulted in elevated expression of cPGES in the brain [7]. Administration of IL-1 into the mouse cortex via intraparenchymal microinjection led to an increase in PGE_2, which was accompanied by elevated expression of cPGES as well as of COX-2 and mPGES-1 with different kinetics [85]. In pregnant female mice, cPGES was strongly detected in the stroma underlying the luminal epithelium surrounding the implanting blastocyst at the implantation site and in decidualized cells under artificial decidualization, whereas only a basal level of cPGES was observed in the control horn [86]. The mouse cPGES gene promoter is GC-rich and contains many Sp1 sites, but lacks an obvious TATA box motif [87]. At present, the transcriptional regulation of cPGES is still unknown.

Co-transfection and antisense experiments indicated that cPGES is capable of converting COX-1-, but not COX-2-derived PGH_2 to PGE_2 in cells, particularly during the immediate PGE_2-biosynthetic response elicited by Ca^{2+}-evoked stimuli [7]. Localization of cPGES in the cytosol may allow coupling with proximal COX-1 in the ER in preference to distal COX-2 in the perinuclear envelope, although other regulatory mechanisms could be involved.

3.3. *Possible in vivo functions of cPGES*

Functional coupling of cPGES with COX-1 suggests that the functions of cPGES *in vivo* overlap significantly, if not entirely, with COX-1. COX-1-derived PGE_2 may be involved in gastrointestinal protection, reproduction, and some neuronal functions, leading to acute pain in the periphery and affecting the central nervous system, which in turn affects daily activities. To elucidate the *in vivo* function of cPGES, cPGES-deficient mice were developed [12–14], but cPGES deletion studies have not been particularly informative in addressing the roles of cPGES-derived PGE_2, because cPGES-deficient mice were perinatal-lethal with poor lung development, delayed skin maturation, and growth retardation (Fig. 4). The lung phenotype was consistent with a defective glucocorticoid response and with p23 acting as a co-chaperone for the glucocorticoid

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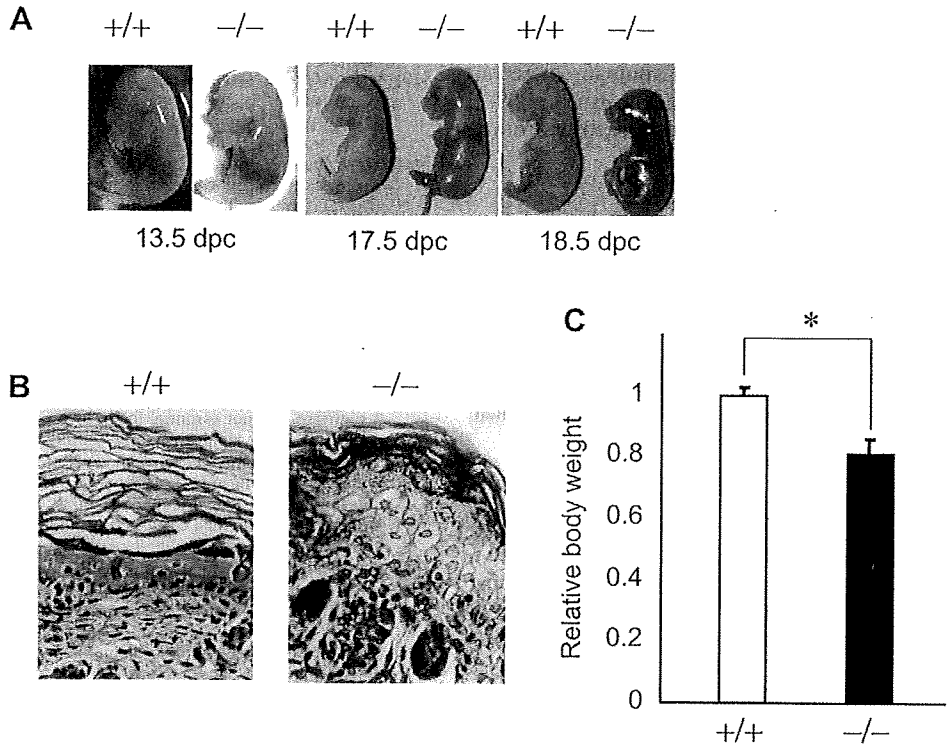

Fig. 4. Phenotypes observed in cPGES/p23 knockout mice. A. Side view of embryos from the same littermates. After 17.5 dpc, cPGES/p23 knockout embryos began to exhibit glossy and sticky skin. B. HE staining of skin sections of wild-type (+/+) and cPGES/p23 knockout (−/−) neonates. Original magnification: 400×. Compared with wild-type mice, the epidermis of knockout mice displayed remarkable abnormalities, including spinous layer hyperplasia and cornified envelope deformation. Such epidermal malformation causes the loss of the epidermal water barrier, leading to neonatal death. C. Growth retardation of cPGES/p23 knockout embryos. Relative whole-body weight of wild-type (+/+) and cPGES/p23 knockout (−/−) embryos between 17.5 and 18.5 dpc. Data were analyzed by Student's *t*-test. The results are expressed as means ± standard error, with $p = 0.05$ as the limit of significance. For details, see Ref. [13].

receptor/Hsp90 complex. In fibroblasts and tissues from cPGES-deficient mice, the expression of glucocorticoid-responsive genes, as well as the glucocorticoid transcriptional activation of reporter plasmids, was reduced [12,14]. Defective nuclear translocation of the glucocorticoid receptor in cPGES-deficient fibroblasts was also observed. Although PGE_2 levels were reduced in lung and other tissues in cPGES-null mice, primary fibroblasts from these mice showed increased, rather than decreased, PGE_2 production [13,14].

Interestingly, the reduction of cPGES in rat spinal cord with intrathecal application of cPGES antisense oligonucleotides reduced nociceptive behavior in zymosan-evoked thermal hyperalgesia and in the formalin assay [88]. These results indicate that cPGES plays an important role in mediating early responses during spinal nociceptive processing. Further studies using tissue-specific cPGES-knockout mice will be needed to clarify the *in vivo* roles of cPGES-derived PGE_2.

4. mPGES-2

4.1. Biochemical properties of mPGES-2

The third PGES, mPGES-2, was initially purified from the microsomal fraction of bovine heart [89], and cDNAs encoding human and monkey homologs were subsequently identified [9]. mPGES-2 is a 41 kDa protein consisting of 378–385 amino acids, which is structurally distinct from mPGES-1; moreover, unlike mPGES-1, mPGES-2 does not exclusively depend on GSH for its catalytic activity [9]. mPGES-2 has an N-terminal hydrophobic domain, followed by a glutaredoxin/thioredoxin homology region, in which the consensus thioredoxin homology sequence of Cys^{110}-X-X-Cys^{113} is present. A mutagenesis study indicated that Cys^{110}, but not Cys^{113}, is essential for the enzymatic activity [90]. The V_{max} and K_m values for PGH_2 of the purified recombinant mPGES-2 are about 3.3 μmol/min/mg and 28 μM, respectively [9]. The recombinant enzyme is activated by various SH-reducing reagents, such as dithiothreitol, GSH, and β-mercaptoethanol in the order of decreasing effectiveness. In addition, the reduced form of lipoic acid (dihydrolipoic acid) serves as one of the natural activators of mPGES-2 in the cells [90].

Crystallization of mPGES-2 reveals that it forms a dimer and attaches to the lipid membrane by anchoring the N-terminal section [91]. Two hydrophobic pockets connected to form a V shape are located at the bottom of a large cavity. The geometry suggests that the SH of Cys^{110} in the glutaredoxin/thioredoxin-like domain is most likely the catalytic site of mPGES-2. PGH_2 fits well into the V-shaped pockets and its endoperoxide moiety interacts with the SH of Cys^{110}. The fold of mPGES-2 is quite similar to that of GSH-dependent hematopoietic PGD synthase, except for the two large loop sections.

4.2. Expression and cellular function of mPGES-2

mPGES-2 is synthesized as a Golgi membrane-associated protein, and the proteolytic removal of the N-terminal hydrophobic domain leads to the formation of a mature cytosolic enzyme [92]. When transfected in several cell lines, mPGES-2 is coupled with both COX-1 and COX-2, leading to PGE_2 production [92]. Since the mature mPGES-2 exists as an N-terminally truncated cytosolic form, mPGES-2 can function as a cytosolic enzyme rather than a membrane-bound enzyme.

The transcript for mPGES-2 is more abundantly distributed in the brain, heart, skeletal muscle, kidney, and liver than in other tissues [9]; this differs from the expression profile of mPGES-1. mPGES-2 expression is rather constitutive in various cells and tissues and is not elevated appreciably during inflammation or tissue damage.

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This suggests that these two enzymes are not always redundant but rather exhibit tissue-specific functions. However, a considerable increase in mPGES-2 expression is observed in human colorectal cancer, in which mPGES-1 is also overexpressed [92]. Also, mPGES-2 expression is dramatically increased in mouse bone marrow stromal cells in accordance with *in vitro* culture [93].

4.3. Possible in vivo functions of mPGES-2

Very recently, mPGES-2-deficient mice showed no specific phenotype and no alteration in PGE_2 levels in several tissues (including liver, kidney, heart, and brain) or in LPS-stimulated macrophages [15]. These results suggest that mPGES-2 is not involved in PGE_2 synthesis under the physiological and pathological conditions tested thus far. However, the possibility of tissue-specific or particular pathological roles of mPGES-2 has not yet been ruled out.

5. Concluding remarks

It has become apparent that there are three PGES enzymes in mammalian cells and that they display distinct functional coupling with upstream COX enzymes. Distinct PGES enzymes may control the spatial and temporal production of PGE_2 in different pathophysiological aspects in particular tissues and cells. Therefore, it is very important to understand the functions specific to each PGES. Although COX-2 inhibitors have reduced gastrointestinal toxicity as compared with traditional NSAIDs, there are also some adverse effects associated with this new group of drugs. Specific inhibition of COX-2 alters the balance between platelet-derived thromboxane A_2 and endothelium-derived PGI_2, leading to increases in the risk of thrombosis due to altered vascular tone [94]. Thus, more selective modulation of the prostanoid pathway appears to be desirable. Theoretically, a pharmacologic blockade of PGES could decrease pathological PGE_2 production while sparing other prostanoids including PGI_2 and thromboxane A_2. Further investigation into the biochemical properties, transcriptional regulation, and *in vitro* and *in vivo* functions of each PGES enzymes may illuminate the potential utility of clinically targeting PGES.

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Microsomal prostaglandin E synthase-1 deficiency attenuates neuronal cell death and mitigates Alzheimer's disease-like pathology in a mouse model

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Epidemiological and clinical studies have suggested that long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) moderate the onset or progression of Alzheimer's disease (AD). As NSAIDs inhibit cyclooxygenase (COX) activity, it was suggested that prostaglandin E_2 (PGE_2), a major end-product of COX, may have a pathogenic role in AD. However, the involvement of PGE synthase (PGES), a terminal enzyme downstream of COX, has not been fully elucidated. We here found that among three PGES isozymes, only microsomal PGES-1 (mPGES-1) expression is induced and associated with β-amyloid (Aβ) plaques in cerebral cortex from human AD patients and Tg2576 mice, a transgenic AD mouse model. Treatment of primary mouse cerebri neuronal cells with Aβ induced mPGES-1 gene expression and PGE_2 production, following significant apoptotic cell death. To evaluate potential roles of mPGES-1 in the development of AD, we have used mPGES-1 deficient mice and found that deletion of mPGES-1 attenuates Aβ-induced neuronal cell death *in vitro* and mitigates AD-like pathology in Tg2576 mice. Accumulation of microglia around senile plaques was reduced and learning impairments were attenuated in mPGES-1 deficient Tg2576 mice. These results indicated that mPGES-1 is induced in AD brains and then plays a role in AD pathology. Blockage of mPGES-1 could form the basis of a novel therapeutic strategy for AD, which may represent a safer approach than therapies involving NSAIDs.

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder of the elderly and is characterized clinically by a progressive memory loss, as well as other cognitive impairments. The neuropathological hallmarks of AD include abundant deposits of β-amyloid peptide (Aβ) fibrils in senile plaques (SPs), accumulation of abnormal tau protein filaments in neurofibrillary tangles (NFTs), and extensive neuronal degradation and loss (1). AD brains also exhibit a number of pathological abnormalities, including a profound loss of synapses, profuse reactive gliosis, microglial activation, and inflammatory process.

Many epidemiological studies have shown that long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) reduces the risk of developing AD and delays its onset (2, 3). It has been demonstrated that neurodegeneration of AD is accompanied by inflammatory reactions, which is indicated by accumulation of microglia and astrocytes around senile plaques and elevated levels of inflammatory cytokines, chemokines, proteases, and reactive oxygen species (4, 5). The preventing effect of NSAIDs has also been modeled in transgenic mice expressing mutant forms of amyloid precursor protein (APP) and presenilin 1 (PS1), and in these models, NSAIDs significantly reduce amyloid deposition and microglial activation (6, 7). However, the mechanism by which NSAIDs may affect these or other pathophysiogical processes relevant to AD is unclear.

NSAIDs inhibit the activity of cyclooxygenase (COX), which catalyzes the conversion of arachidonic acid to prostaglandin H_2 (PGH_2), the precursor of the five PGs: PGE_2, PGD_2, PGI_2, $PGF_{2\alpha}$, and thromboxane A_2 (TXA_2). Of the two COX isoforms, COX-1 is expressed

constitutively in most tissues and is generally responsible for the production of PGs that control normal physiological functions, while COX-2 is inducible in response to mitogens, cytokines, and cellular transformation (8). Although it remains uncertain as to how NSAIDs prevent the development of AD, several studies have shown that elevated COX-2 expression is present in cerebral cortex of AD brain and correlate with amyloid plaque density and NFTs (9-13). Significantly, levels of PGE_2, which is the main product of COX, have been found to be elevated in cerebrospinal fluid of AD patients (14), suggesting that PGE_2 signaling may function in the development of AD.

PGE_2 is synthesized from COX-derived PGH_2 by the action of PGE synthase (PGES) (15). Thus far, three PGES enzymes, microsomal PGES (mPGES)-1, mPGES-2, and cytosolic PGES (cPGES), have been identified (16). Among these PGES isozymes, mPGES-1 is induced by proinflammatory stimuli and down-regulated by anti-inflammatory glucocorticoids as in the case of COX-2, and is functionally coupled with COX-2 in marked preference to COX-1 (16, 17). Induction of mPGES-1 expression has been observed in various systems in which COX-2-driven PGE_2 has been implicated, such as rheumatoid arthritis, febrile response, reproduction, bone metabolism, and cardiovascular function (18). Furthermore, Satoh et al. reported that Aβ induced mPGES-1 expression in rat astrocytes (19). However, little is known about the involvement of mPGES-1, downstream enzyme of COX-2, in AD.

In the present study, to examine the expression and distribution of mPGES-1 in AD brain, we performed immunohistochemical analysis using brain tissues from human AD patients and Tg2576 mice. Tg2576 mice harbor the Swedish mutation of APP and exhibit some of the

pathological features of the AD brain (20). We here found that among three PGES isozymes, only mPGES-1 expression is induced and associated with Aβ plaques in cerebral cortex from both human AD patients and Tg2576 mice. The induction of mPGES-1 was also observed in Aβ-treated primary mouse nerve cells. Furthermore, we have used mPGES-1 deficient mice (21, 22) to evaluate potential roles of mPGES-1 in the development of AD, and found that mPGES-1 deficiency attenuates Aβ-induced neuronal cell death *in vitro* and mitigates AD-like pathology in Tg2576 mice. These results indicated that mPGES-1 is induced in AD brains and then plays a role in AD pathology.

Results

High Expression of mPGES-1 in Brains of Human AD and Tg2576 Mice. First, immunohistochemical studies were performed in human brain tissues from 18 patients. Positive immunoreactivity for mPGES-1 and -2 was observed in all tested brain tissues, but definite immunostaining with cPGES antibody was not found.

As shown in Fig. 1A, even in case of normal control (NC) hippocampus, dense immunostaining with the mPGES-1 antibody was observed in the cytoplasm and neurites in the CA2-3 (CA: cornu ammonis) region, but less intense staining in the CA4 and sparse positive staining in the CA1 were found. No positive staining was detected in the dentate gyrus and subiculum. In AD hippocampus, a similar staining pattern could be seen with much more intense labeling. Very clear cytoplasmic staining with many areas of neurite labeling was seen in the CA2-3 (Fig. 1A), and to a lesser extent in the CA4, and sparse but definite staining was also seen

in the CA1 and subiculum regions. No staining was seen in the dentate gyrus. In non-AD hippocampus, the intensity of the labeling was equivalent to that of NC (data not shown). There were no differences in the staining of representative tissue samples of frontal and parietal lobes of NC, AD and non-AD cases, except mild to very weak cytoplasmic staining of the cortices, and no labeling of the white matter. Quantitative image analysis confirmed that the expression of mPGES-1 was more extensive in AD brains than in NC and non-AD brains (Fig. 1B). Furthermore, we performed double-immunostaining using antibodies that recognized mPGES-1 and phosphorylated tau or Aβ to investigate whether the mPGES-1 expression was implicated in the pathogenesis of AD. As the results, we found that mPGES-1 was expressed with dystrophic neurites around senile Aβ plaques in AD brains (Fig. 1C).

On the other hand, with the anti-mPGES-2 antibody, the hippocampus of all groups exhibited consistent staining pattern of similar intensity (data not shown). Through the CA to the subiculum, glial and neuronal cytoplasmic staining was uniformly observed with many areas of neurite labeling. Mild cytoplasmic staining was seen in the dentate gyrus. Some glial neurite labeling extended to vessels to form a perivascular pattern, suggesting that the positive staining was of astrocytes. In the frontal and parietal lobes, neuronal and glial staining was seen in the cortical region, and glial staining in the white matter was observed. Some perivascular staining was also seen. With the anti-cPGES antibody, there was no definite staining of any tissue samples of hippocampus, frontal and parietal lobes from NC, AD and non-AD groups (data not shown). The expression levels of mPGES-2 and cPGES in AD brains were similar to those in the other brains. Neither mPGES-2 nor cPGES expressions were associated with Aβ plaques.

We next examined the expression and distribution of mPGES-1 in brains of old-aged (16 months old) Tg2576 mice. mPGES-1-immunoreactive cells were observed in neuronal cells in limited areas of brains of Tg2576 and age-matched control mice. The expression level of mPGES-1 was higher in Tg-2576 mice than control mice (Fig. 1D). Immunostaining with anti-human Aβ antibody (82E1) revealed Aβ plaques in the hippocampus and cortex of the old-aged Tg2576 mice, although Aβ immunostaining was not observed in the age-matched control mice. The mPGES-1 expression was found around the Aβ plaques in Tg2576 mice, and staining was particularly evident in areas of dense Aβ plaques (Fig. 1E). Furthermore, double-immunostaining using antibodies specific to each cell marker showed that the mPGES-1-stained cells were found to be glial fibrillary acidic protein (GFAP)-positive reactive astrocytes (Fig. 1F). In our study, GFAP-immunoreactive cells were observed in both Tg2576 and controls, and astrocytes that were intensely immunoreactive for GFAP were found surrounding the Aβ plaques in Tg2576 mice. On the other hand, the mPGES-2- or cPGES- immunoreactive cells were observed in whole brain of Tg2576 and control mice (data not shown). Neither mPGES-2 nor cPGES was found to be associated with Aβ plaques.

These results indicated that among the three PGES isozumes, only mPGES-1 expression is induced and associated with the pathogenesis in both human AD patients and Tg2576 mice.

Induction of mPGES-1 Expression in Aβ–treated Neuronal Cells *in Vitro*. As Satoh et al. reported that Aβ induced mPGES-1 expression in rat astrocytes *in vitro* (19), we next used Aβ fragment 31-35 ($A\beta_{31\text{-}35}$) as Aβ peptide and examined whether mPGES-1 is induced in mouse

primary neuronal cells by Aβ. It has been reported that $A\beta_{31-35}$ was induce cytotoxic and pro-apoptotic effects in several neuronal cells (23, 24), although it does not exhibit aggregation phenomena (25). Primary cerebral neuronal cells were prepared from the cerebri of 16.5-day-old mouse embryos and then treated by Aβ. As shown in Fig. 2A, RT-PCR analysis revealed that mPGES-1 mRNA was barely detectable in the untreated cells but markedly increased by Aβ without alteration of COX-2 expression. Production of PGE_2 was also increased with increase in the mPGES-1 expression 48 h after Aβ treatment (Fig. 2B). On the other hand, neither induction of mPGES-1 nor increment in PGE_2 production was observed in Aβ-treated neuronal cells derived from mPGES-1 deficient mice. These results indicated that mPGES-1 contributes to the formation of PGE_2 in Aβ-treated neuronal cells.

Furthermore, to determine the cell type expressing mPGES-1, double-immunostaining of mPGES-1 and GFAP or microtubule-associated protein-2 (MAP-2) was carried out. The immunoreactivity of mPGES-1 was slightly but significantly detected near the nuclear membrane in untreated cells and its positive signals were enhanced after Aβ treatment (Fig. 2C). The mPGES-1-immunoreactive signals were observed in GFAP-positive astrocytes as well as a portion of MAP-2-positive neurons.

mPGES-1 Deficiency Attenuates Aβ-induced Neuronal Cell Death *in Vitro*. The effect of mPGES-1 deficiency on Aβ-induced neuronal cell death *in vitro*. As shown in Fig. 3A, the cell number of primary neuronal cells derived from wild-type mice was increased during the study period. After 48 h of Aβ treatment, the increase in cell number was significantly repressed. Double-immunostaining of GFAP and MAP-2 revealed that both of GFAP-positive astrocytes and

MAP-2-positive neurons proliferated during the period and both growth was suppressed by Aβ (Fig. 3B). On the other hand, proliferation of neuronal cells derived from mPGES-1 deficient mice was not suppressed by Aβ treatment. Conversely, Aβ modestly increased the growth of the mPGES-1 deficient cells (Fig. 3).

As it was shown that Aβ induces apoptosis in cultured neuronal cells (23-25), we next examined the effect of mPGES-1 deficiency on Aβ-induced apoptosis by TUNEL staining. As shown in Fig. 4A, the number of TUNEL-positive apoptotic cells were increased when wild-type neuronal cells were treated with Aβ for 72 h. On the other hand, apoptosis of mPGES-1 deficient neuronal cells was not induced by Aβ treatment. PGE_2 also could not induce apoptosis in mPGES-1 deficient cells as well as Aβ, but combination of Aβ and PGE_2 could induce apoptosis (Fig. 4B). These results indicated that Aβ-induced mPGES-1 dependent PGE_2 production is necessary but not sufficient for Aβ-induce neuronal cell apoptosis.

mPGES-1 Deficiency Mitigates the AD-like Pathology in Tg2576 Mice. To determine whether mPGES-1 contributes to Aβ-dependent behavioral deficits *in vivo*, we bred Tg2576 ($APPsw^{+/-}$) mice, which develop Aβ plaques starting at 9 to 10 months of age and impair spatial memory without neuron loss (20), with mPGES-1 deficient mice to generate $APPsw^{+/-}$/mPGES-$1^{+/-}$ (as Tg2576 mice), $APPsw^{+/-}$/mPGES-$1^{-/-}$ (as mPGES-1 deficient Tg2576 mice), $APPsw^{-/-}$/mPGES-$1^{+/-}$ (as control mice), and $APPsw^{-/-}$/mPGES-$1^{-/-}$ mice (as mPGES-1 deficient mice). Morris water-maze tests were performed using these mice at the age of 10 to 11 months. During the 5-day period of training trials, the latency in finding a target platform of all groups of mice was decreased (data not shown). Over the entire period, the Tg2576 mice did not

differ from the control mice in latency, distance of swimming and swimming speed. To measure spatial learning and memory retention, we next performed the probe trials on the day after the 5-day training was finished. As shown in Fig. 5A, in the probe trial, only APPsw$^{+/-}$/mPGES-1$^{+/-}$ mice significantly failed to favor the target platform location compared to control APPsw$^{-/-}$/mPGES-1$^{+/-}$ mice. On the other hand, APPsw$^{+/-}$/mPGES-1$^{-/-}$ mice tended to swim around the target location as well as control mice, indicating that mPGES-1 deficiency improved target crossing in Tg2576 mice.

Furthermore, we found that histopathological features of Tg2576 mice were also affected by mPGES-1 deficiency. As shown in Fig. 5B, around senile plaques of brain derived from APPsw$^{+/-}$/mPGES-1$^{+/-}$ mice at the age of 10 to 11 months, mPGES-1 protein was expressed and reactive atrocytes and swollen microglia were accumulated. On the other hand, in APPsw$^{+/-}$/mPGES-1$^{-/-}$ mice, only a small numbers of reactive astrocytes and no swollen microglia were observed around senile plaques, although there was no significant difference in Aβ plaque deposition between APPsw$^{+/-}$/mPGES-1$^{+/-}$ and APPsw$^{+/-}$/mPGES-1$^{-/-}$ mice. These results suggested that mPGES-1 deficiency might suppress inflammatory reactions occurred in brains of Tg2576 mice.

Discussion

In the present study, we found that mPGES-1 is induced in cerebral cortex from AD patients and Tg2576 mice *in vivo* (Fig. 1) and Aβ-treated mouse cerebri nerve cells *in vitro* (Fig. 2), and that mPGES-1 deficiency attenuates Aβ-induced apoptosis of neuronal cells *in vitro* (Figs. 3 and 4)

and mitigates AD-like pathology in Tg2576 mice (Fig. 5). It has been shown that mPGES-1 is up-regulated in many tissues by proinflammatory cytokines or growth factors (18). The increase in mPGES-1 expression is strongly correlated with the induction of COX-2 in many conditions, and mPGES-1 is shown to be preferentially coupled with COX-2 activity to increase the pathologic production of PGE_2 (16-18). However, these are not always the case. Sandee et al. reported that mPGES-1 was overexpressed and involved in PGE_2 production in both COX-1 and COX-2 deficient cells (27). We here also found that mPGES-1 mRNA was markedly increased by Aβ without alteration of COX-2 expression in mouse neuronal cells (Fig. 2A). Several studies have shown that COX-2 expression is increased in cerebral cortex of AD brain (9-13), but interestingly, decreased expression of COX-2 in end-stage AD has also been reported (28). In addition to this study, Chaudhry et al. recently reported that mPGES-1 levels were elevated in western blots of middle frontal gyrus tissue extracts from AD patients relative to age matched controls (29). mPGES-1 may play more critical roles in pathogenic PGE_2 production in AD brains than COX-2.

Our results indicated that mPGES-1-derived PGE_2 might be involved in Aβ-induced apoptosis of neuronal cells *in vitro* (Figs. 3 and 4). PGE_2 exerts its biological actions through its binding to four specific receptor subtypes known as EP1, EP2, EP3 and EP4 (30). Studies using EP2 deficient mice had shown that among these four EPs, EP2 that is present on microglia, plays an important role in Aβ-activated neurotoxicity (31, 32). Shie et al. used primary cultures of wild-type neurons and microglia from either wild-type or EP2 deficient mice, and found that Aβ-treated wild-type microglia enhanced Aβ-induced apoptosis of wild-type neurons but

Aβ-treated EP2 deficient microglia could not (31). Aβ-induced expression of inducible nitric oxide synthase (iNOS) that can cause neurotoxicity was also reduced in EP2 deficient microglia. Furthermore, Liang et al. showed that deletion of EP2 in APPswe-PS1ΔE9 mice, one of AD model mice, resulted in marked reduction in age-dependent lipid peroxidation in brain tissues (32). These two reports indicated that activation of EP2 by PGE_2 leads to an increase in the production of microglial superoxides and neurotoxins and then increased production of reactive oxygen species promotes lipid peroxidation and injury in neurons. We also found that deletion of mPGES-1 reduced the Aβ-induced generation of 8-isoprostane, a lipid peroxide, in a mixed culture of neurons, astrocytes and microglia (data not shown). mPGES-1-derived PGE_2 produced by astrocytes or neurons might act on microglial EP2 and promote oxidative damage in AD brain.

It has also been shown that PGE_2-EP2 signaling shows bidirectional effects enhancing Aβ-induced neuronal cell death and protecting neuronal cells against Aβ-induced cell death (33). Although PGE_2 and EP2 agonists have neurotoxic effects (34), lower concentrations of PGE_2 and EP2 or EP4 agonists were neuroprotective against Aβ toxicity (33). It was noteworthy that mPGES-1 deficient neuronal cells did not grow without Aβ during the study period (Fig. 3). These results suggested that small amount of mPGES-1-derived PGE_2 might be involved in neuronal cell maintenance and growth. We further found that Aβ did not suppress the growth of mPGES-1 deficient cells but conversely increased it. It was reported that Aβ induced arachidonic acid release by activation of phospholipase A_2 in primary neuronal cells (35). In mPGES-1 deficient cells, Aβ might enhance some arachidonate metabolites other than PGE_2, which stimulate neuronal cell growth. Further studies were needed to reveal the mechanism how Aβ

enhanced the growth of mPGES-1 deficient neuronal cells.

Although unlike AD patients, severe neuronal cell death is not observed, Tg2576 mice develop Aβ senile plaques starting at 9 to 10 months age and impair spatial memory (20). As shown in Fig. 5, we examined the effect of mPGES-1 deficiency on AD-like pathology in Tg2576 mice and found that deletion of mPGES-1 reduced accumulation of microglia and reactive astrocytes around senile plaques without affecting Aβ plaque deposition. Learning impairments in water maze probe trial were also attenuated in mPGES-1 deficient mice. It has been suggested that microglia might play different roles at different time points in the progression of AD pathogenesis (36). Early microglia accumulation, which begins before formation of visible Aβ deposits, might be beneficial. Microglia recruited at early stages of AD phagocytose and clear Aβ and, hence, protect the brain from the toxic effects of Aβ. As the disease progresses, however, and persistent production of proinflammatory cytokines, microglia lose their protective phenotype and their ability to keep up with Aβ deposition and become dysfunctional and unable to clear all of the Aβ (37). In addition to down regulating Aβ clearance, the products of activated microglia are neurotoxic and, hence, promote neuronal degeneration. Our results indicated that mPGES-1-derived PGE_2 positively regulates activation and migration of microglia after Aβ deposition in AD brain and then exacerbates AD pathology.

It was noteworthy that mPGES-1 deficiency did not affect Aβ plaque deposition. Studies using EP2 or EP4 deficient mice showed that both PGE_2 receptors are involved in cleavage of APP and Aβ deposition by (32, 38). mPGES-2- or cPGES-derived PGE_2 might regulate Aβ production from APP and promote AD progression in collaboration with

mPGES-1-derived PGE_2.

In conclusion, our results indicated that mPGES-1 is induced in AD brains and then plays a critical role in AD pathology. Although epidemiological and clinical studies have suggested that long-term use of NSAIDs moderate the onset or progression of AD, their intake is frequently associated with gastrointestinal side effects. Because COX expressed in gastrointestinal mucosa is mainly COX-1, in recent years efforts focused on the development of highly selective COX-2 inhibitors with an improved gastric tolerability profile. However, severe cardiovascular adverse reactions challenged the initial enthusiasm in this new class of anti-inflammatory drugs. Blockage of mPGES-1 could form the basis of a novel therapeutic strategy for AD, which may represent a safer approach than therapies involving NSAIDs.

Materials and Methods

Animal Experiments. Mouse studies were approved by the Institutional Animal Care and Use Committee of Fukuoka University and Showa University. Tg2576 mice ($APPsw^{+/-}$, C57BL/SJL), which contained the APP gene with the human Swedish mutations (K670N/M671L), were obtained from Taconic. For immunohitochemical analysis, brains were obtained from four Tg2576 mice and four age-matched control mice (C57BL/SJL) of 16 months of age. mPGES-$1^{-/-}$ mice were described previously (21). For *in vitro* analysis, we crossed mPGES-$1^{-/-}$ mice (21) with Balb/c mice. For *in vivo* analysis, we crossed Tg2576 ($APPsw^{+/-}$) mice with mPGES-$1^{-/-}$ mice (21) to generate $APPsw^{+/-}$/mPGES-$1^{+/-}$ and $APPsw^{-/-}$/mPGES-$1^{+/-}$ mice. Then, these mice were again

crossed with mPGES-$1^{-/-}$ mice to generate APPsw$^{+/-}$/mPGES-$1^{+/-}$ (as Tg2576 mice), APPsw$^{+/-}$/mPGES-$1^{-/-}$ (as mPGES-1 deficient Tg2576 mice), APPsw$^{-/-}$/mPGES-$1^{+/-}$ (as control mice), and APPsw$^{-/-}$/mPGES-$1^{-/-}$ mice (as mPGES-1 deficient mice).

Human Tissues. Brains from 9 AD cases (average age 85.8±9.5, range 68-98), 5 non-AD cases including individuals with Parkinson's disease and cerebrovascular disorders (average age 78.6±8.2, range 68-90) and 4 cases without neurological disorders (average age 89.3±4.6, range 83-94) defined as normal control were selected from the Choju Medical Institute Brain Bank of Fukushimura Hospital. We obtained informed consent from all subjects. The diagnosis of AD was confirmed in all cases by neuropathological evidence of loss of temporo-parietal neurons and the presence of SPs and NFTs. The control cases were without neurological signs or symptoms and no pathological changes were detected in their brains on post mortem examination.

Immunohistochemistry. For preparation of human brain tissues, about 7 mm thick transverse tissue blocks of the frontal, parietal, and hippocampal regions were fixed in 10% formalin solution and embedded in paraffin until processed for histological examination. For preparation of murine brain tissues, mice were anesthetized with ether, and then using peristaltic pump, perfused with saline followed by 4% paraformaldehyde in PBS. Their brains were removed from their skulls and immersed in 4% paraformaldehyde in PBS at 4°C overnight. The brains were then dehydrated, degreased with ethanol, and embedded in paraffin. Using a microtome, tissues were cut into 5 μm thick sections that were mounted onto glass slides and stored.

Brain tissue sections were deparaffinized in xylene, hydrated in an ascending ethanol and then autoclaved in 10 mM citrate buffer (pH 6.0) for 20 min at 120°C for antigen retrieval. After

cooling at room temperature, the sections were treated for 30 min with 0.3% H_2O_2 to eliminate endogenous peroxidase activity, and then treated with 5% skim milk in PBS for blocking for 30 min at room temperature. After washing PBS-Tween, the sections were incubated with each specific primary antibody (cPGES (diluted 1:500, Cayman, Ann Arbor, MI), mPGES-1 (1:500, Cayman) or mPGES-2 (1:500, Cayman)) overnight at 4℃. Following incubation with the primary antibodies, the sections were treated with the appropriate biotinylated secondary antibody (1:1000, Jackson Immuno Research, West Grove, PA) for 2 hr at room temperature, followed by incubation in avidin-biotinylated horseradish peroxidase complex (1:1000, Vector, Burlingame, CA) for 1 hr at room temperature. The sections were then washed three times with Tris-HCl (pH 7.6) for 5 min each time, and peroxidase labeling was visualized by incubation with a solution containing 0.01% 3,3'-diaminobenzidine (Dojindo, Kumamoto, Japan), 0.05 M imidazole (Wako, Osaka, Japan), 0.00015% H_2O_2 and 0.6% nickel ammonium sulfate (Sigma, St. Louis, MO) in 0.05 M Tris-HCl pH 7.6. At the same time, positive control sections were examined under a microscope to monitor the efficiency of the immunostaining. After dehydration, the sections were cover-slipped using HSR liquid (Sysmex, Kobe, Japan). The results of immunostaining were recorded as digital images, and analyzed by image analysis software (WinRoof, ver. 5.6.1, Mitani Co. Ltd., Osaka, Japan). Illuminated pixels were statistically analyzed by Dunnett's two-tailed test. A P value less than 0.05 was considered statistically significant.

For double immunostaining, sections were treated at the end of the first incubation cycle with 0.5% H_2O_2 for 30 min. The second cycle was carried out similarly to the first, expect that nickel ammonium sulfate was omitted from the DAB solution. A monoclonal antibody to