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Prostaglandin I₂ Attenuates Prostaglandin E₂-Stimulated Expression of Interferon γ in a β -Amyloid Protein- and NF- κ B-Dependent Mechanism

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Pu Wang*, Pei-Pei Guan*, Xin Yu, Li-Chao Zhang, Ya-Nan Su & Zhan-You Wang

Cyclooxygenase-2 (COX-2) has been recently identified as being involved in the pathogenesis of Alzheimer's disease (AD). However, the role of an important COX-2 metabolic product, prostaglandin (PG) I₂, in AD development remains unknown. Using mouse-derived astrocytes as well as APP/PS1 transgenic mice as model systems, we firstly elucidated the mechanisms of interferon γ (IFN γ) regulation by PGE₂ and PGI₂. Specifically, PGE₂ accumulation in astrocytes activated the ERK1/2 and NF- κ B signaling pathways by phosphorylation, which resulted in IFN γ expression. In contrast, the administration of PGI₂ attenuated the effects of PGE₂ on stimulating the production of IFN γ *via* inhibiting the translocation of NF- κ B from the cytosol to the nucleus. Due to these observations, we further studied these prostaglandins and found that both PGE₂ and PGI₂ increased A β ₁₋₄₂ levels. In detail, PGE₂ induced IFN γ expression in an A β ₁₋₄₂-dependent manner, whereas PGI₂-induced A β ₁₋₄₂ production did not alleviate cells from IFN γ inhibition by PGI₂ treatment. More importantly, our data also revealed that not only A β ₁₋₄₂ oligomer but also fibrillar have the ability to induce the expression of IFN γ *via* stimulation of NF- κ B nuclear translocation in astrocytes of APP/PS1 mice. The production of IFN γ finally accelerated the deposition of A β ₁₋₄₂ in β -amyloid plaques.

Alzheimer's diseases (AD) is the most common cause of dementia in aged people and is characterized clinically by cognitive decline and pathologically by the accumulation of β -amyloid protein (A β) and hyperphosphorylation of tau in the brain¹. It has been generally accepted that neuroinflammation is involved in A β deposition and tau phosphorylation, which contribute to the progression of AD^{2,3}. Although the mechanisms of neuroinflammation in AD have not yet been elucidated, cyclooxygenase-2 (COX-2) has been suggested as having a potential role in neuroinflammation. This is due to its metabolic products, the prostaglandins (PGs), which include PGE₂, PGD₂ [and its dehydration end product, 15-deoxy- Δ ^{4,5}-PGJ₂ (15d-PGJ₂)], PGI₂, PGF_{2 α} and TXA₂⁴. Among these PGs, both PGE₂ and PGI₂ are potential mediators of inflammation^{5,6}. For example, PGE₂ is involved in all processes leading to the classic signs of inflammation: redness, swelling and pain⁹. Pain results from the action of PGE₂ on peripheral sensory neurons and on central sites within the spinal cord and the brain⁷. Apart from PGE₂, PGI₂ signaling facilitated joint inflammation in a mouse model of collagen-induced arthritis, while the administration of a PGI₂ antagonist reduced pain and inflammation in rodent models of hyperalgesia and chronic arthritis⁸. In contrast to the seemingly pro-inflammatory properties of PGI₂, there is still debate about its effects in certain conditions². For example, PGI₂ has been studied as a potentially important suppressor of allergen-induced inflammation². Thus, the effects of PGI₂ on inflammatory reactions of peripheral tissues are still uncertain rather than neuroinflammation.

Although we could not find direct evidence that demonstrates the relationship between PGs and neuroinflammation, a growing body of research reveals that both PGE₂ and PGI₂ has the ability to regulate the synthesis of cytokines⁹. For example, our prior works demonstrated that PGE₂ has ability to stimulate the expression of IL-1 β

College of Life and Health Sciences, Northeastern University, Shenyang 110819, P. R. China. *These authors contributed equally to this work. Correspondence and requests for materials should be addressed to P.W. (email: wangpu@mail.neu.edu.cn) or Z.-Y.W. (email: wangzy@mail.neu.edu.cn)

in A172 cells¹⁰. In addition, TNF- α was also stimulated in PGE₂-stimulated SH-SY5Y cells¹¹. In astrocytes, PGE₂ also showed its stimulatory effects on the expression of IL-6¹² and IFN γ ^{13,14}. Similar to PGE₂, PGI₂ analogues including iloprost and treprostinil treatment induced IL-10 expression but suppressed TNF- α expression in human myeloid dendritic cells⁹. Additionally, Wahlstrom *et al.*¹⁵ reported that when compared to a placebo treatment, the administration of the PGI₂ analogue epoprostenol significantly decreased C-reactive protein (CRP) and generally decreased IL-6 levels in patients with severe traumatic brain injury. Following from this observation, Schuh *et al.*¹⁶ reported that the early induction of PGI₂ at the site of traumatic injury resulted in the aggregation of IL-1 β -expressing macrophages as a critical cause of neuropathic pain. Apart from interleukins and TNF- α , Strassheim *et al.*¹⁷ reported that PGI₂ inhibits interferon γ (IFN γ)-stimulated cytokine expression in human monocytes. However, the regulatory mechanisms between PGI₂ and IFN γ , including the role of PGI₂ in regulating the expression of IFN γ during the course of AD development are often not studied.

Although little is known about the relationship between PGE₂/I₂ and IFN γ , IFN γ has already been suggested to regulate the pathogenesis of AD¹⁸. For example, IFN γ treatment activates the promoter of BACE-1 in human U373MG astrocytoma cells¹⁹. Additionally, IFN γ stimulates β -secretase expression and sAPP β production in mouse astrocytes²⁰. Yamamoto *et al.*²¹ also found that IFN γ regulates amyloid plaque (AP) deposition in Swedish mutant APP transgenic mice. Apart from β -secretase, it has also been reported that IFN γ production has the ability to accelerate γ -secretase cleavage of APP²² by upregulating the expression of presenilin 2 (PS2) in human neuronal cells²³. When considered together, these data prompted us to investigate the roles of PGE₂ and PGI₂ in regulating the expression of IFN γ during the course of AD development.

To understand the relationship between PGs and IFN γ , we first delineated the signaling pathway of IFN γ upregulation in APP/PS1 mice. Specifically, we demonstrated that PGE₂ induction at the early stage of AD stimulates the expression of IFN γ via A β ₁₋₄₂-dependent NF- κ B-activating pathways. In contrast, PGI₂ attenuated the effects of PGE₂ on stimulating the expression of IFN γ by depressing NF- κ B nuclear translocation. Although PGI₂ also has the ability to enhance the production of A β ₁₋₄₂, A β ₁₋₄₂ could not alleviate IFN γ inhibition from PGI₂ treatment. In addition, not only A β oligomers but also A β fibrils have ability to stimulate the expression of IFN γ , which is responsible for sustaining high levels of IFN γ during the course of AD development. Reciprocally, IFN γ accumulation in or secretion from astrocytes accelerates the A β deposition in APs. Therefore, PGE₂ and PGI₂ have opposing effects on IFN γ expression, which is responsible for accelerating A β ₁₋₄₂ deposition in APs during the course of AD development.

Materials and Methods

Reagents. PGI₂, PGE₂, A β ₁₋₄₂ and the inhibitors NS398, U0126, and KT5720 were obtained from Sigma-Aldrich Corp (St. Louis, MO, USA). Antibodies against β -actin, ERK1/2, p-ERK1/2 (Thr 202/Tyr 204), NF- κ B, p-NF- κ B (Ser 536), p-NF- κ B (Ser 276), I κ B, IFN γ , BACE-1, PS1, PS2, GFAP and human A β were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). sAPP α and sAPP β antibodies were obtained from IBL International Corp. (Toronto, ON, Canada). The human IFN γ and IFN γ enzyme immunoassay kits were obtained from Raybiotech, Inc. (Norcross, GA, USA). Human or mouse A β ₁₋₄₂ ELISA kits were obtained from Invitrogen (Carlsbad, CA, USA). ERK1/2, p65 and scramble siRNA were obtained from Cell Signaling Technology, Inc. (Danvers, MA, USA). The chromatin immunoprecipitation (ChIP) EZ-ChIP kit was purchased from Upstate Biotechnology. All reagents for the qRT-PCR and SDS-PAGE experiments were purchased from Bio-Rad Laboratories. All other reagents were from Invitrogen (Carlsbad, CA, USA) unless otherwise specified.

Transgenic mice and treatments. The female wild type (WT) or APP/PS1 transgenic mice [B6C3-Tg (APP^{swe}, PSEN1dE9) 85Dbo/J (Stock Number: 004462)] were obtained from The Jackson laboratory (Bar Harbor, ME, USA)²⁴. Genotyping was performed at 3–4 weeks after birth. The mice were housed in a controlled environment under a standard room temperature, relative humidity and 12-h light/dark cycle with free access to food and water. Mice were randomly separated into several groups and each group contains 10 mice. Mice at 6 months of age were injected (i.c.v) with PGE₂ (2 μ g/5 μ l) or PGI₂ (2 μ g/5 μ l) in the absence or presence of A β antibody (1 μ g/5 μ l) or A β oligomers (1 μ g/5 μ l) for 24 h before determining the expression of IFN γ . In select experiments, WT mice at 6 months of age were injected with 5 μ l CSF that was collected from APP/PS1 mice at 6 months of age [in the absence or presence of A β antibody (1 μ g/5 μ l)] at 24 h prior to IFN γ gene expression studies. In separate experiments, the WT mice were injected (i.c.v) with A β oligomers (1 μ g/5 μ l) or fibrils (1 μ g/5 μ l) at 24 h prior to IFN γ gene expression studies. In distinct experiments, IFN γ (10 ng/20 μ l/d) was nasally administered to 3-months-old WT or APP/PS1 mice for 7 days, 3 months or 6 months before determining the A β deposition in APs. The general health and body weights of animals were monitored every day. The brains of animals from the different groups were collected under anesthesia and perfusion as previously described²⁵.

A β ₁₋₄₂ preparation. The methods for preparing A β oligomers or fibrils had been described previously²⁶⁻²⁸. In brief, freeze-drying A β ₁₋₄₂ protein (Stock Number: A9810, Sigma, St. Louis, MO, USA) was initially monomerized by dissolving it to a final concentration of 1 μ g/ μ l in 100% hexafluoroisopropanol (HFIP) and the solution was aliquoted in sterile eppendorf tubes. HFIP was then evaporated under vacuum and the peptide was stored at -20 °C before reconstituted. For preparing A β ₁₋₄₂ oligomers, the peptide was initially resuspended in dimethylsulfoxide (DMSO) to 20 μ g/ μ l with water bath ultrasonication for 10 min and the solution was then diluted to a final concentration of 0.2 mg/ml in phenol red-free F-12 media, and incubated at 4 °C for 24 h. To prepare A β ₁₋₄₂ fibrils, A β ₁₋₄₂ was resuspended in sterile Milli Q water and incubated at 37 °C for 1 week before use.

Intracerebroventricular injection (i.c.v). NS398, PGE₂, PGI₂, A β , or anti-human A β or vehicle (PBS) solutions were injected (i.c.v) into WT or APP/PS1 transgenic mice as previously described²⁵. In selected experiments, the WT mice were injected (i.c.v) with the CSF of APP/PS1 mice. Briefly, stereotaxic injections were placed

at the following coordinates from the bregma: mediolateral: -1.0 mm; anteroposterior: -0.22 mm; and dorsoventral: -2.8 mm. Following injections, each mouse recovered spontaneously on a heated pad. The reliability of injection sites was validated by injecting trypan blue dye (Invitrogen) into separate cohorts of mice and observing staining in the cerebral ventricles. Twenty-four hours after injection, mice were harvested under anesthesia and perfusion as previously described²⁵.

Organotypic slice culture of brain tissue. Brain tissues were freshly collected from WT C57BL/6 mice at 6 months of age. Serial sections ($400\text{-}\mu\text{m}$ thick) were cut using a chopper without fixation. The tissue sections were immediately cultured in DMEM/high glucose medium with 10% fetal bovine serum (FBS). In a separate set of experiments, the tissues were grown in serum-free medium for an additional 24 h before incubation with A β oligomers or fibrils, as previously described²⁵. The tissue sections were fixed and immunostained with IFN γ antibody by an immunohistochemical staining kit (Invitrogen, Carlsbad, CA, USA).

Luciferase assays and live animal imaging. The experiments were performed as previously described²⁶. The D1A cells that were transfected with an IFN γ promoter were pre-seeded in one side of a ventricle. PGI₂, PGE₂ or vehicle (PBS) solutions were then injected (i.c.v) into the other side of ventricle. At different time intervals, mice were anesthetized and injected (i.c.v) with luciferin into the cerebral ventricle, which was preseeded with D1A cells. The scan was performed exactly after 5 min of luciferin introduction. All images were analyzed using Bruker *in vivo* imaging systems (MS FX PRO, Carestream, U.S.A.).

Cell culture. Mouse astrocyte D1A and neuroblastoma n2a cells were grown (37°C and 5% CO₂) on 6-cm tissue culture dishes (10^6 cells per dish) in appropriate medium. In a separate set of experiments, the cells were grown in serum-free medium for an additional 24 h before incubation with inhibitors in the absence or presence of PGI₂ or PGE₂, as previously described^{10,25}.

ChIP Assay. This assay was performed using the EZ ChIP kit following the manufacturer's instructions (Upstate Biotechnology) as described previously^{27–30}. Forward (F) and reverse (R) primers for IFN γ promoter amplification by qPCR are as follows: F-CGTTGACCCTGAGTGATTTG and R-GTTTCCTTCGACTCCTTG.

Quantitative real-time PCR (qRT-PCR). qRT-PCR assays were performed with the MiniOpticon Real-Time PCR detection system (Bio-Rad) using total RNA and the GoTaq one-step Real-Time PCR kit with SYBR green (Promega) and the appropriate primers as previously described³¹. The GenBank accession number and forward and reverse primers for mouse GAPDH and BACE-1 are provided in our previous publications^{10,32,33}: mouse IFN γ (NM_008337.3) F-CACGGCACAGTCATGAAAG, R-ATCAGCAGCGACTCCTTTTC; GFAP (NM_001131020) F-AATGCTGGCTTCAAGGAGAC, R-CTCCAGCGATTCAACCTTTC; PS1 (NM_008943) F-GCTTGTTAGGCGCTTTAGTG, R-CATCTGGGCATTCTGGAAGT; PS2 (NM_011183) F-AAGAACGGGCAGCTCATCTA, R-TCCAGACAGCCAGGAAGAGT. The gene expression values were normalized to those of GAPDH.

Western blot analysis. Tissues or cells were lysed in radio-immune precipitation assay buffer (25 mM Tris-HCl [pH 7.6], 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, and 0.1% SDS) that contained a protease inhibitor cocktail (Pierce Chemical Company). The protein content of the cell lysates was determined using the bicinchoninic acid (BCA) protein assay reagent (Pierce Chemical Company). The total protein lysates ($4\mu\text{g}$) were separated using SDS-PAGE, transferred to a membrane, and probed with a panel of specific antibodies. In general, primary and secondary antibody was diluted in TBST by the ratio of 1:2000 and 1:5000, respectively. Each membrane was only probed with one antibody. β -actin was used as a loading control. The membrane was visualized by ECL. All western hybridizations were performed at least in triplicate using a different cell preparation each time.

Immunohistochemistry. Brain tissues were collected from WT or APP/PS1 transgenic mice. In selected experiments, brain tissues were collected after injection (i.c.v) of PGI₂ ($2\mu\text{g}/5\mu\text{l}$) or PGE₂ ($2\mu\text{g}/5\mu\text{l}$). Serial sections ($5\text{-}\mu\text{m}$ thick) were cut using a paraffin microtome (Leica, RM2235, Germany). Sections were first rehydrated in a graded series of ethanol and submerged in 3% hydrogen peroxide to eliminate endogenous peroxidase activity. The activity of astrocytes was determined by staining GFAP using an immunohistochemical staining kit, following the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA).

Immunofluorescence. Brain tissues were collected from WT or APP/PS1 transgenic mice. In selected experiments, brain tissues were collected after injection (i.c.v) of PGI₂ ($2\mu\text{g}/5\mu\text{l}$) or PGE₂ ($2\mu\text{g}/5\mu\text{l}$). Serial sections ($10\text{-}\mu\text{m}$ thick) were cut using a cryostat (Leica, CM1850, Germany). Slides were stained with IFN γ or A β antibody with Alexa Fluor 555 or 488 secondary antibodies (Cell Signaling Technology, Inc., Danvers, MA, USA) before observing under confocal microscopy (Leica, TCS-SP8, Leica).

Measurement of the IFN γ concentration in the culture medium or the brain of mice. The IFN γ levels in the media of both control and pharmacologically treated cells or the brain of mice were determined using IFN γ enzyme immunoassay kits following the manufacturer's instructions. The total protein used for ELISA was used as a loading control, and the results are expressed as pg of IFN γ per mg of total protein.

Transfection. Cells were transfected with 100 nM of an ERK1/2- or p65-specific siRNA oligonucleotide. In control experiments, the cells were transfected with 100 nM scrambled siRNA. The transfected cells were allowed to recover for at least 12 h in growth medium and then incubated overnight in serum-free medium before extraction.

Animal committee. All animals were handled according to the care and use of medical laboratory animals (Ministry of Health, Peoples Republic of China, 1998) and all experimental protocols were approved by the Laboratory Ethics Committees of College of Life and Health Sciences of Northeastern University.

Human brain samples. Human brain samples were obtained from New York Brain Bank, serial numbers P535-00 (normal), TT4263 (early stage of AD, the patient is 73-years-old man who was diagnosed as a mild AD patient), T4308 (middle stage of AD, the patient is 86-years-old man who was diagnosed as moderate AD patient), T4339 and T4304 (late stage of AD, the patients are 88-years-old woman and 84 years-old woman who were diagnosed as severe and end stage of AD patients).

Statistical analysis. All data are represented as the mean \pm S.E. of at least three independent experiments. The statistical significance of the differences between the means was determined either using Student's *t*-test^{10,25}.

Results

IFN γ is markedly upregulated in APP/PS1 transgenic mouse brain. Due to previous studies suggesting that IFN γ plays a critical role in the pathogenesis of AD²¹, we evaluated the expression levels of IFN γ in AD patients and APP/PS1 transgenic mice at 6 or 9 months of age. As shown in Fig. 1A, IFN γ immunostaining was progressively induced during the course of AD development. Interestingly, the morphology analysis demonstrated that positive staining of IFN γ translocated from neurons to astrocytes. In line with these observations in AD patients, IFN γ immunostaining was highly enhanced in the cerebral cortex and dentate gyrus (DG) region of the hippocampus of APP/PS1 mice at 6 months of age when compared to WT C57BL/6 mice (Fig. 1B). These data reveal that IFN γ is upregulated with the development/progression of AD. To further confirm this finding, we examined the mRNA and protein levels of IFN γ in these APP/PS1 Tg mice. In agreement with the immunostaining data, our results demonstrated the upregulation of IFN γ mRNA and protein levels in the cerebral cortex and DG region of the hippocampus (Fig. 1C,D). In addition, we found that IFN γ was also stimulated in APP/PS1 mice at 9 months of age (Fig. 1E). Similarly, mRNA and protein levels of IFN γ were sustained above the basal levels (Fig. 1F,G). These observations indicate the possible role of A β aggregation in IFN γ stimulation.

NS398 treatment attenuates the expression of IFN γ in APP/PS1 transgenic mice. Because COX-2 expression was elevated at the early stage of AD and was associated with A β deposition³⁴, we studied whether COX-2 inhibition by NS398 downregulates the expression of IFN γ . We intranasally administered NS398 (50 μ g/kg/d) to WT or APP/PS1 mice for 6 months prior to sacrifice. The results demonstrated that NS398 administration decreased the mRNA and protein expression of IFN γ (Fig. 1H). To further validate the above results, we injected (i.c.v) APP/PS1 mice at 6 months of age with NS398 (2 μ g/5 μ l). After 24 h, the brains of mice were collected and the expression of IFN γ was determined. The mRNA and protein expression of IFN γ was highly induced in the APP/PS1 mice, which was blocked by NS398 injection (Fig. 1I). These observations clearly indicate that COX-2 elevation stimulated the expression of IFN γ in APP/PS1 transgenic mice.

PGE₂ upregulates the expression of IFN γ , whereas PGI₂ downregulates the expression of IFN γ . Because NS398 treatment markedly decreased the expression of IFN γ in APP/PS1 mice at 6 months of age (Fig. 1H,I), we sought to determine the roles of COX-2 metabolic products, including PGE₂ and PGI₂, in regulating the expression of IFN γ following intracerebroventricular injection. It is evident that PGE₂ (2 μ g/5 μ l) injection (i.c.v) stimulated the expression of IFN γ in the dentate gyrus (DG) region of hippocampus of mice (Fig. 2A). The mRNA and protein levels of IFN γ were detected using qRT-PCR and ELISA. The results showed that PGE₂ injection (i.c.v) increased the expression of IFN γ in the cerebral cortex of WT mice (Fig. 2C). To further verify the key role of PGE₂ in upregulating the expression of IFN γ *in vivo*, we combined i.c.v injection with live animal imaging. As described in Fig. 2E, D1A cells that were transfected with the IFN γ promoter constructs were pre-seeded in the left lateral ventricle of WT mice at 6 months of age, whereas PGE₂ (2 μ g/5 μ l) was injected into the right ventricle of the same mice. After 24 h, luciferin was injected (i.c.v) into the side of cerebral ventricles of APP/PS1 Tg mice, which was pre-seeded with D1A cells before live animal imaging. The results showed that PGE₂ increased the luciferase activity of the IFN γ promoter (Fig. 2E). To understand if the increased production of IFN γ was a result of microglia or astrocyte activation, we determined the activity of astrocytes following i.c.v injection of PGE₂. The results demonstrated that astrocytes were markedly stimulated by PGE₂ injection (Fig. 2G,H).

To further understand the roles of COX-2 metabolic products in IFN γ regulation, we similarly injected (i.c.v) PGI₂ into the ventricles of 6-months-old APP/PS1 mice. In contrast to PGE₂, PGI₂ injection (i.c.v) decreased the positive staining of IFN γ in the cerebral cortex of APP/PS1 transgenic mice at 6 months of age (Fig. 2B). mRNA and protein levels of IFN γ were assessed using qRT-PCR and western blots. Similar results were obtained as in IHC assays (Fig. 2D). Additionally, PGI₂ treatment actively alters the transcriptional activity of the IFN γ promoter and synthesis in live animals, as observed by live animal imaging (Fig. 2F). We then sought to understand the role of PGI₂ in regulating the expression of IFN γ through the activity of astrocytes by quantifying their activity following injecting (i.c.v) with PGI₂. As expected, the activity of astrocytes was suppressed by PGI₂ injection (i.c.v) (Fig. 2I). In addition, PGI₂ treatment suppressed the expression of GFAP in cerebral cortex and hippocampus (Fig. 2J). These observations not only demonstrated the opposing roles of PGE₂ and PGI₂ in regulating the expression of IFN γ , but also indicated the possible roles of astrocytes in expressing IFN γ .

NF- κ B nuclear translocation plays an important role in mediating the effects of PGE₂ and PGI₂ in regulating the expression of IFN γ in astrocytes. As PGE₂ and PGI₂ demonstrated antagonistic effects on regulating the expression of IFN γ , we next determined the mechanism of IFN γ regulation by PGE₂ and PGI₂. Using D1A cell culture, we found that PGE₂ treatment induced the phosphorylation of ERK1/2 without

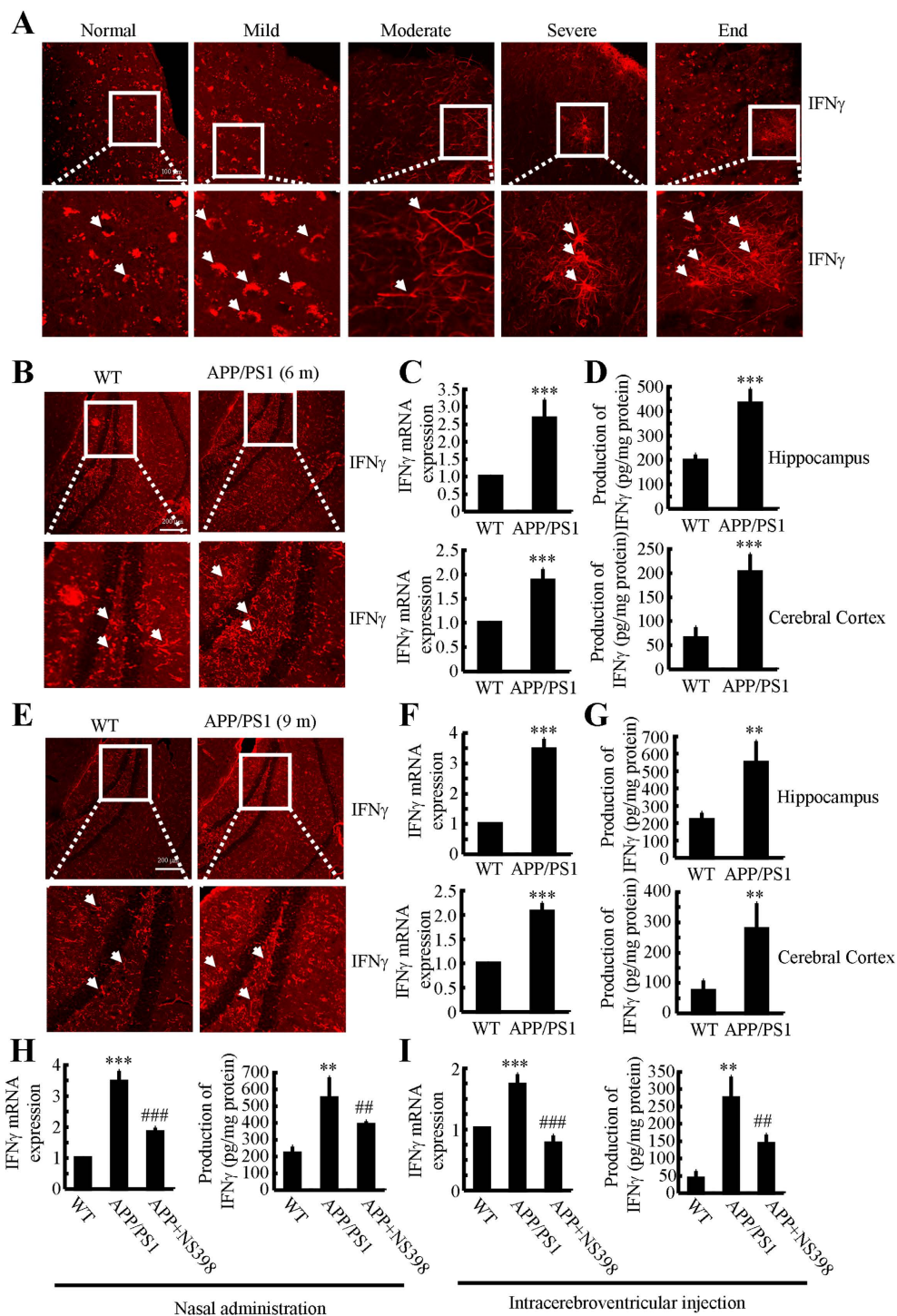


Figure 1. NS398 treatment decreases the induction of IFN γ in APP/PS1 mice. (A) The tissue blocks of human brains at different stages of AD were collected by the New York Brain Bank at Columbia University. Free-floating slices (40 μ m) were prepared by cryostat. (B–G) The brains of WT or APP/PS1 transgenic mice at 6 or 9 months of age were collected following anesthesia and perfusion. In select experiments, the APP/PS1 transgenic mice at the age of 3 months received NS398 (50 μ g/kg/d) intranasally for 6 months before brain harvesting (H). In separate experiments, APP/PS1 mice were injected (i.c.v) with NS398 (2 μ g/5 μ l) for 24 h (I). The immunoreactivity of IFN γ was determined by immunohistochemistry using an anti-IFN γ antibody (A, B, E). The arrows demonstrated the positive staining of IFN γ . IFN γ protein and mRNA levels were determined by IFN γ enzyme immunoassay kits and qRT-PCR, respectively (C–I). Total amounts of protein and GAPDH served as an internal control. The data represent the means \pm S.E. of at least three independent experiments. * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ with respect to WT control. # $p < 0.05$; ## $p < 0.01$ and ### $p < 0.001$ compared to APP/PS1 alone.

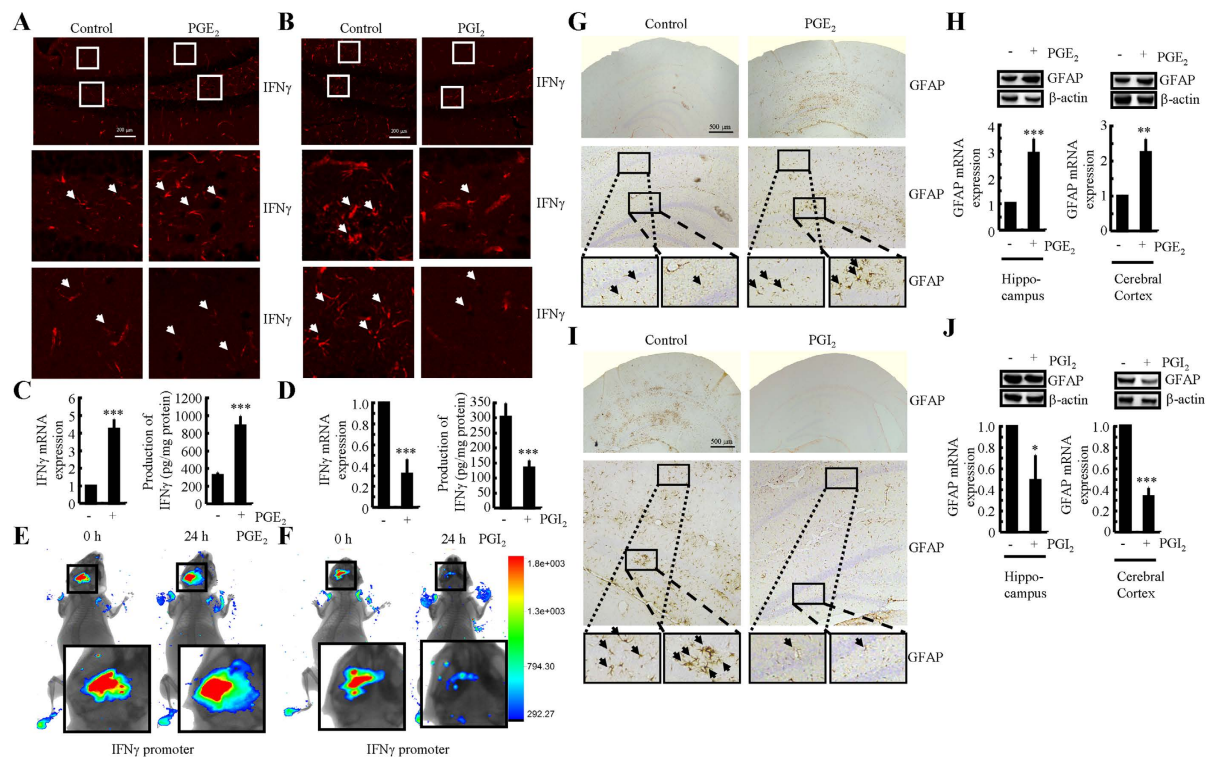


Figure 2. Antagonistic effects of PGE₂ and PGI₂ on regulating the expression of IFN γ in WT or APP/PS1 transgenic mice. The WT or APP/PS1 C57BL/6 mice at the age of 6 months were injected (i.c.v.) with PGE₂ (2 μ g/5 μ l) or PGI₂ (2 μ g/5 μ l). The brains were then collected and sectioned after 24 h (A,B,G,I). In select experiments, one side of the cerebral ventricle was injected with PGE₂ (2 μ g/5 μ l) or PGI₂ (2 μ g/5 μ l), and the other side of the cerebral ventricle was injected (i.c.v.) with D1A cells, which was pre-transfected with the IFN γ promoter (E,F). The immunoreactivity of IFN γ was determined by immunofluorescence staining using an anti-IFN γ antibody (A,B). Luciferase activities from the different groups of mice were measured using live animal imaging system (E,F). The activities of astrocytes were determined by immunohistochemistry with anti-GFAP (G,I). mRNA and protein levels of IFN γ and GFAP were determined by qRT-PCR, western blot and IFN γ enzyme immunoassay kits, respectively (C,D,H,J). Total amounts of GAPDH, β -actin and protein served as an internal control. The data represent the means \pm S.E. of at least three independent experiments. * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ with respect to PBS (–) or vehicle-treated controls.

altering the total protein levels of ERK1/2 in D1A (Fig. 3A). To further elucidate the potential role of ERK1/2 in regulating the expression of IFN γ , we treated D1A cells with the pharmacological ERK1/2 inhibitor U0126 (10 μ M) in the absence or presence of PGE₂ (10 μ M). Incubation of D1A cells with U0126 (10 μ M) not only suppressed the PGE₂-induced phosphorylation of ERK1/2 but also reversed the PGE₂-stimulated IFN γ synthesis (Fig. 3A). To eliminate any potential non-specific effects of the pharmacological ERK1/2 inhibitor U0126, we conducted experiments with D1A cells that were transfected with an siRNA oligonucleotide sequence that was specific for ERK1/2. ERK1/2 knockdown and scramble control cells were treated with PGE₂ (10 μ M) or vehicle control for 48 h. ERK1/2 knockdown markedly reversed the stimulatory effects of PGE₂ on the mRNA and protein expression of IFN γ in D1A cells (Fig. 3B).

To identify the mechanism of the transcriptional upregulation of IFN γ by PGE₂, we determined the possible involvement of transcriptional factors in this process. Due to our previous observations¹⁰, we found that PGE₂ treatment stimulates the phosphorylation of NF- κ B at both Ser 536 and Ser 276 sites in D1A cells (Fig. 3C). The activation of NF- κ B was blocked by U0126 treatment (Fig. 3C), which indicates the potential contribution of NF- κ B in regulating IFN γ synthesis. To decipher the role of NF- κ B in mediating IFN γ synthesis, we next treated D1A cells with the PKA inhibitor KT5720 (5 μ M) in the absence or presence of PGE₂ (10 μ M). The results demonstrated that KT5720 treatment reversed the effects of PGE₂-induced expression of IFN γ via suppressing the phosphorylation of NF- κ B at the sites of Ser 536 and Ser 276 in D1A cells (Fig. 3C). The reason for using KT5720 to inhibit NF- κ B is because NF- κ B located downstream of PKA to exert biological function^{35,36}. To eliminate any non-specific effects of KT5720 on the activity of NF- κ B, we conducted experiments using cells that were transfected with an siRNA oligonucleotide that was specific for the NF- κ B p65 subunit. The efficacy of the p65 knockdown was assessed through quantifying p65 protein levels in D1A cells (Fig. 3D upper panel). p65 knockdown reversed the stimulatory effects of PGE₂ on the mRNA and protein expression of IFN γ in D1A cells (Fig. 3D lower panel). In particular, we found that PGE₂ increased NF- κ B translocation to the nucleus by decreasing the amount

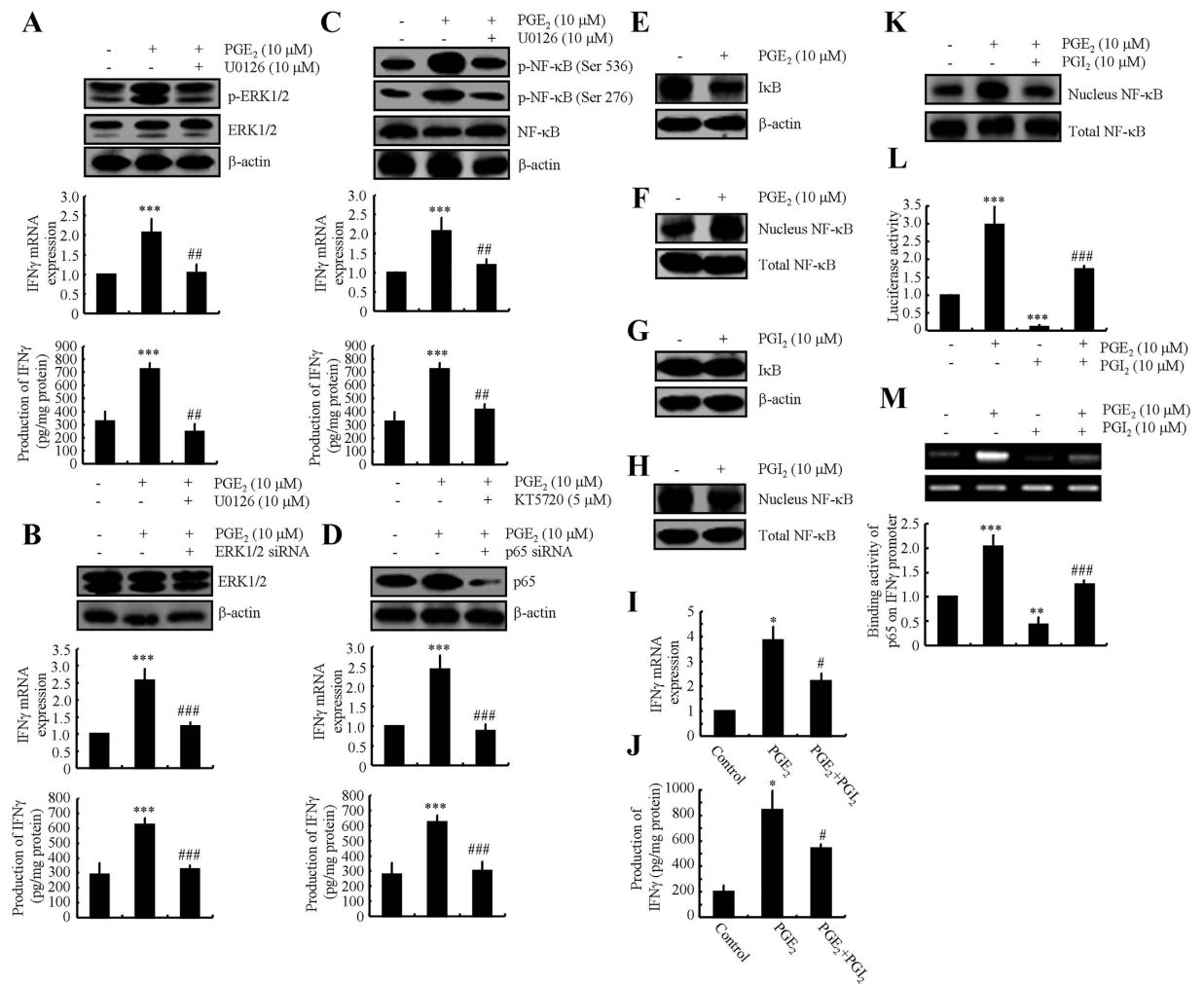


Figure 3. Critical roles of NF- κ B activity in regulating IFN γ expression by PGE $_2$ - and PGI $_2$ -stimulated D1A cells. Mouse astrocyte D1A cells were treated with PGE $_2$ (10 μ M) in the absence or presence of the ERK1/2 inhibitor U0126 (10 μ M) (A,C upper panel), KT5720 (5 μ M) (C lower panel) for 24 h before extracting protein or mRNA (A,C,E,F). In select experiments, the cells were transfected with ERK1/2 or p65 siRNA before incubation with PGE $_2$ (10 μ M) for 24 h (B,D). In separate experiments, cells were treated with PGI $_2$ (10 μ M) for 24 h (G,H). In distinct experiments, the cells were treated with PGE $_2$ (10 μ M) in the absence or presence of PGI $_2$ (10 μ M) for 24 h (I–M). Total ERK1/2 (A,B), phosphorylated ERK1/2 levels (A), total NF- κ B (C,D), phosphorylated NF- κ B (C) and total I κ B (E,G) were detected by immunoblotting using specific antibodies. Equal lane loading was demonstrated by the similar intensities of total β -actin. The nuclear and total NF- κ B levels were determined by western blots (F,H,K). IFN γ protein and mRNA levels were determined by IFN γ enzyme immunoassay kits and qRT-PCR, respectively (A–D,I,J). Total amounts of protein and GAPDH served as an internal control. The luciferase activity of the IFN γ promoter was determined by dual luciferase reporter assay kits (L). The binding activity of NF- κ B to the promoter of IFN γ was determined by ChIP assay (M). The data represent the means \pm S.E. of at least three independent experiments. * p < 0.05; ** p < 0.01 and *** p < 0.001 with respect to the vehicle-treated or vector-transfected control. # p < 0.05; ## p < 0.01 and ### p < 0.001 compared to PGE $_2$ -treated alone.

of I κ B in D1A cells (Fig. 3E,F). In contrast, PGI $_2$ decreased nuclear translocation of NF- κ B without affecting the total amount of I κ B in D1A cells (Fig. 3G,H). These data further support the notion that PGE $_2$ and PGI $_2$ have antagonistic effects on the regulation of IFN γ expression in a NF- κ B-dependent manner.

PGI $_2$ attenuates the effects of PGE $_2$ on stimulating the expression of IFN γ . In an effort to validate this hypothesis, we treated D1A cells with PGE $_2$ in the absence or presence of PGI $_2$. The results showed that PGI $_2$ attenuated the effects of PGE $_2$ on stimulating the expression of IFN γ (Fig. 3I). Although PGI $_2$ reduced the expression of IFN γ in PGE $_2$ -injected mice, the level of IFN γ was still above the basal level (Fig. 3I). This observation was then confirmed using ELISA (Fig. 3J). As PGE $_2$ and PGI $_2$ demonstrated opposing effects on the phosphorylation and nuclear translocation of NF- κ B (Fig. 3F, H), we sought to determine whether NF- κ B transcriptionally mediated the effects of PGE $_2$ and PGI $_2$ on regulating the expression of IFN γ in D1A cells. We found that PGI $_2$

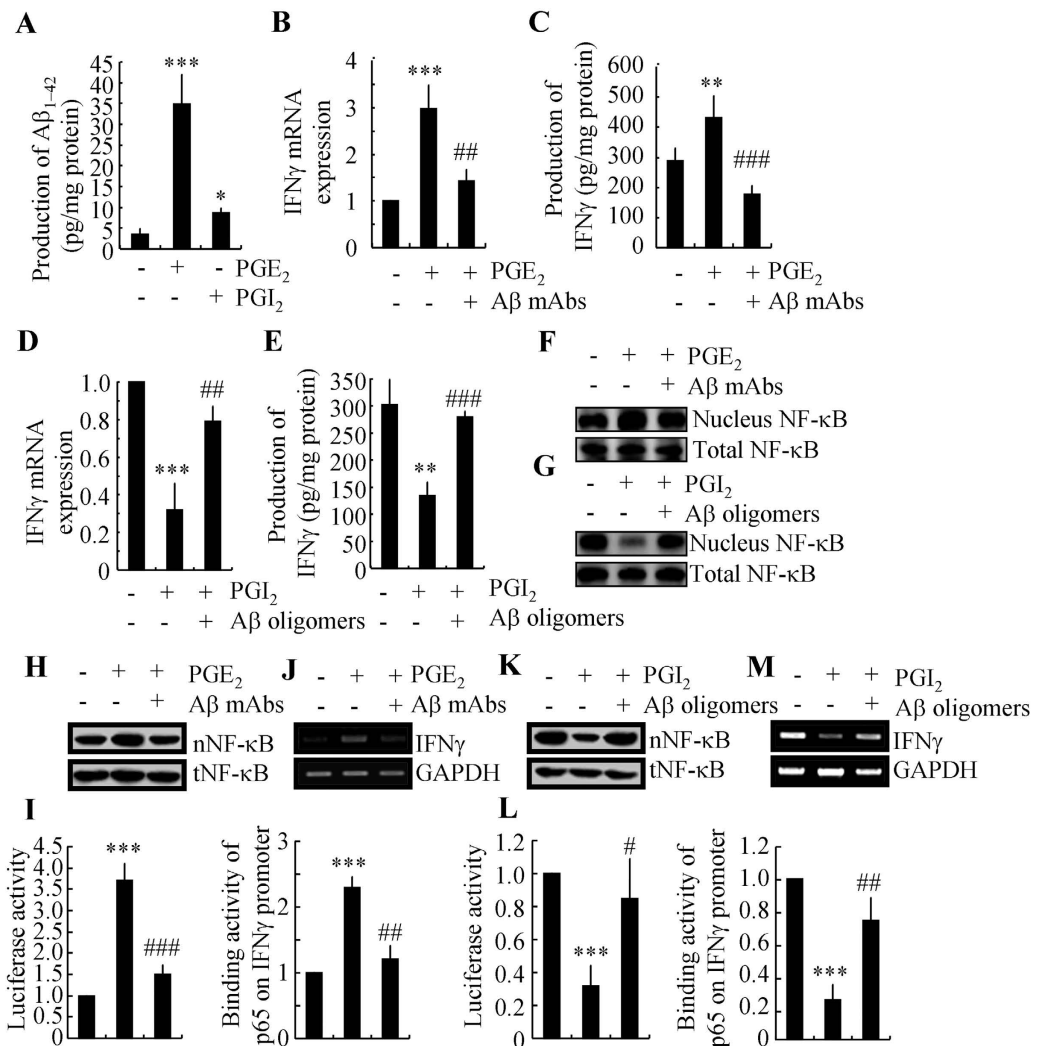


Figure 4. Aβ₁₋₄₂ mediated the antagonistic effects of PGE₂ and PGI₂ on regulating the expression of IFNγ. D1A cells were treated with PGE₂ (10 μM) or PGI₂ (10 μM) for 48 h (A). In select experiments, PGE₂ (2 μg/5 μl) or PGI₂ (2 μg/5 μl) was injected (i.c.v.) into the ventricles of WT C57BL/6 or APP/PS1 mice in the absence or presence of Aβ antibody (1 μg/5 μl) or Aβ₁₋₄₂ oligomers (1 μg/5 μl) for 24 h (B–G). In separate experiments, D1A cells were treated with PGE₂ (10 μM) in the absence or presence of Aβ antibody (1 μg/ml) for 24 h (H–J). In distinct experiments, D1A cells were treated with PGI₂ (10 μM) in the absence or presence of Aβ₁₋₄₂ oligomers (1 μM) (K–M). The production of Aβ₁₋₄₂ was determined by Aβ₁₋₄₂ ELISA kits (A). Total amount of protein served as internal control. IFNγ protein and mRNA levels were determined by IFNγ enzyme immunoassay kits and qRT-PCR, respectively (B–E). Total amounts of protein and GAPDH served as an internal control. The nuclear and total NF-κB levels were determined by western blots (F–K). The luciferase activity of the IFNγ promoter was determined by dual luciferase reporter assay kits (L, L). The binding activity of NF-κB to the promoter of IFNγ was determined by ChIP assay (J, M). The data represent the means ± S.E. of at least three independent experiments. **p* < 0.05; ***p* < 0.01 and ****p* < 0.001 with respect to the vehicle-treated control. #*p* < 0.05; ##*p* < 0.01 and ###*p* < 0.001 compared to PGE₂-treated alone.

attenuated the stimulatory effects of PGE₂ on NF-κB nuclear translocation (Fig. 3K), which is consistent with the level of mRNA transcripts and protein synthesis of IFNγ (Fig. 3I, J). We also found that PGE₂ upregulated the IFNγ promoter activity, whereas PGI₂ downregulated the promoter activity of IFNγ (Fig. 3L). As expected, PGI₂ attenuated the effects of PGE₂ on stimulating the promoter activity of IFNγ (Fig. 3L). These data were further confirmed using chromatin immunoprecipitation assays (Fig. 3M).

Aβ₁₋₄₂ is involved in mediating PGE₂- and PGI₂-regulated IFNγ expression via an NF-κB-dependent mechanism. As Aβ₁₋₄₂ has an essential role in neuroinflammation^{2,3}, we sought to determine the involvement of Aβ₁₋₄₂ in mediating the effects of PGE₂ and PGI₂ on regulating the expression of IFNγ. Interestingly, we found that both PGE₂ and PGI₂ have the ability to stimulate the production of Aβ₁₋₄₂ (Fig. 4A). However, PGI₂ displayed a relatively weak ability to stimulate the production of Aβ₁₋₄₂ when compared

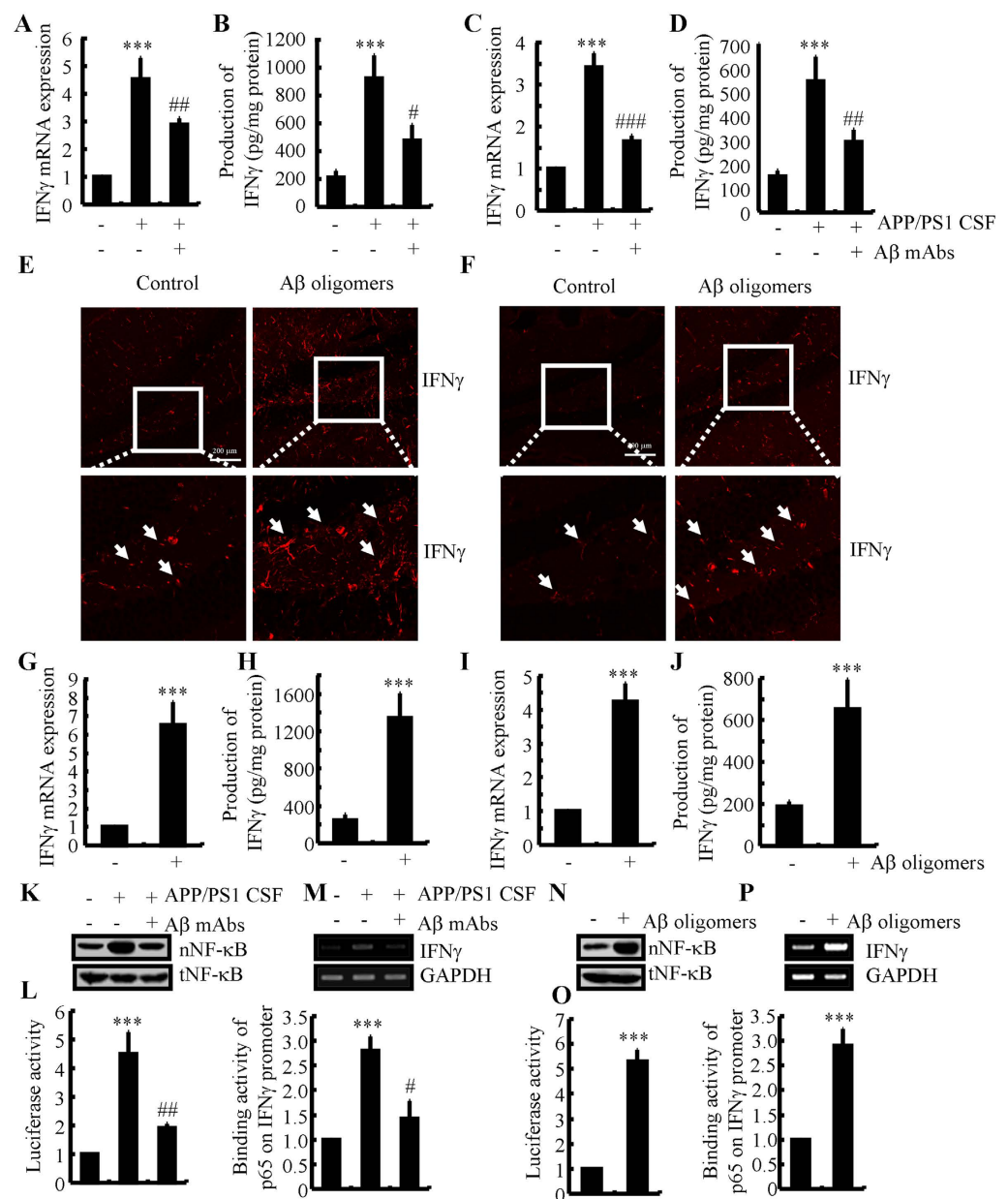


Figure 5. IFN γ upregulation at the early stage of AD was caused by A β oligomers. Cerebrospinal fluid (CSF) of APP/PS1 mice at 6 months of age was collected and then injected (i.c.v.) into wild type C57BL/6 mice in the absence or presence of A β antibody (1 μ g/5 μ l) for two weeks before sacrifice (A,B). In select experiments, D1A cells were treated with CSF of APP/PS1 mice at 6 months of age (1:1000 dilution) in the absence or presence of A β antibody (1 μ g/ml) for 24 h (C,D,K–M). In separate experiments, the wild type C57BL/6 mice at the age of 6 months were injected (i.c.v.) with A β oligomers (2 μ g/5 μ l) for 24 h (E,G,H). In distinct experiments, the slices of 6-month-old WT mice or D1A cells were cultured in A β_{1-42} oligomers (E,I,J,N–P). The immunoactivity of IFN γ was determined by an immunofluorescence assay (E,F). IFN γ protein and mRNA levels were determined by IFN γ enzyme immunoassay kits and qRT-PCR, respectively (A–D,G–J). Total amounts of protein and GAPDH served as an internal control. The nuclear and total NF- κ B levels were determined by western blots (K,N). The luciferase activity of the IFN γ promoter was determined by dual luciferase reporter assay kits (L,O). The binding activity of NF- κ B to the promoter of IFN γ was determined by CHIP assay (M,P). The data represent the means \pm S.E. of at least three independent experiments. * p < 0.05; ** p < 0.01 and *** p < 0.001 with respect to vehicle-treated controls. # p < 0.05; ## p < 0.01 and ### p < 0.001 compared to APP/PS1 CSF-treated alone.

to PGE $_2$. To further understand the role of A β_{1-42} in IFN γ regulation, we injected (i.c.v.) PGE $_2$ (2 μ g/5 μ l) into the ventricles of WT mice in the absence or presence of A β_{1-42} antibody (1 μ g/5 μ l). The results demonstrated that the

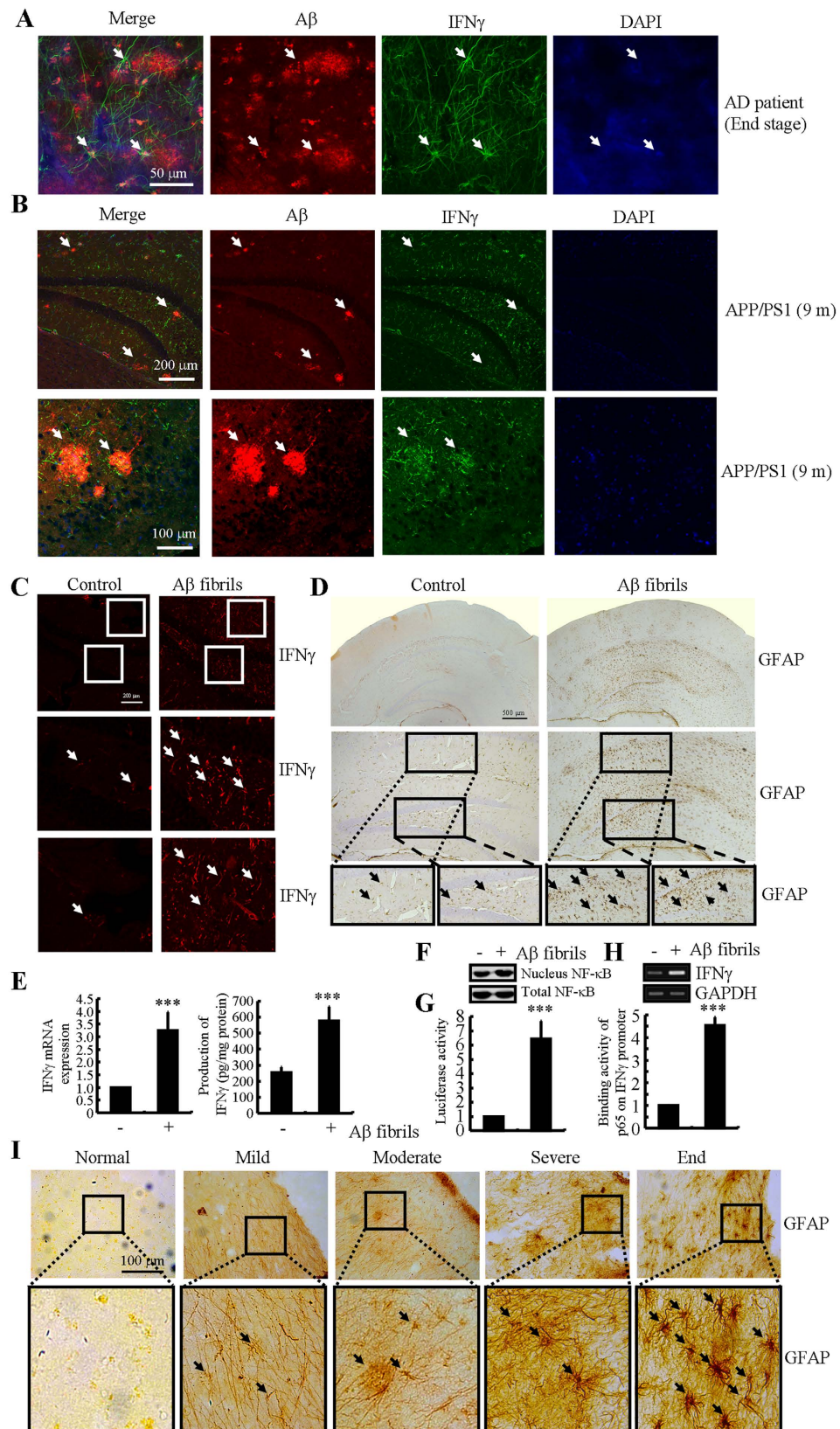


Figure 6. IFN γ upregulation at the late stage of AD was caused by advanced aggregated A β in APs. (A,I) The tissue blocks of human brains at different stages of AD were collected by the New York Brain Bank at Columbia University. Free-floating slices (40 μ m) were prepared by cryostat. (B) The brains of WT or APP/PS1 transgenic mice at 9 months of age were collected after anesthesia and perfusion. In select experiments, brain slices of 6-month-old WT mice were cultured in the absence or presence of A β ₁₋₄₂ fibrils for 24 h (C, D). In separate experiments, D1A cells were incubated with A β fibers for 24 h (E–H). The slices of mouse brains were

double-stained with A β (red) or IFN γ (green) antibodies before being observed under confocal microscopy (A,B). The immunoactivity of IFN γ was determined by an immunofluorescence assay (C). The activity of astrocytes was determined by staining with GFAP (D,I). IFN γ protein and mRNA levels were determined by IFN γ enzyme immunoassay kits and qRT-PCR, respectively (E). Total amounts of protein and GAPDH served as an internal control. The nuclear and total NF- κ B levels were determined by western blots (F). The luciferase activity of the IFN γ promoter was determined by a dual luciferase reporter assay kits (G). The binding activity of NF- κ B to the promoter of IFN γ was determined by a ChIP assay (H). The data represent the means \pm S.E. of at least three independent experiments. * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ with respect to vehicle-treated controls.

A β_{1-42} antibody thoroughly diminished the stimulatory effects of PGE $_2$ on IFN γ expression (Fig. 4B,C). Because PGI $_2$ also increased the production of A β_{1-42} , we then treated the APP/PS1 mice at 6 months of age with PGI $_2$ (2 μ g/5 μ l) in the absence or presence of A β oligomers (1 μ g/5 μ l). The results showed that A β oligomers restore the decreasing expression of IFN γ in PGI $_2$ injected (i.c.v) mice (Fig. 4D,E). As PGI $_2$ stimulates the production of A β_{1-42} , which is responsible for IFN γ synthesis, we sought to understand how elevated PGI $_2$ levels depressed the expression of IFN γ while not stimulating its production. In view of our data showing the opposite effects of PGE $_2$ and PGI $_2$ on NF- κ B nucleus translocation, we further determined NF- κ B mobility in different groups of mice. The results revealed that A β antibody attenuated the PGE $_2$ -induced NF- κ B nucleus translocation, whereas A β oligomers restored the suppressive effects of PGI $_2$ on NF- κ B nucleus translocation (Fig. 4F,G). To further confirm these *in vivo* observations, we treated the D1A cells with PGE $_2$ in the absence or presence of A β_{1-42} antibody. The results demonstrated that A β_{1-42} antibody blocked the effects of PGE $_2$ on stimulating the NF- κ B nuclear translocation (Fig. 4H). However, A β_{1-42} oligomers administration increased the NF- κ B nuclear translocation in PGI $_2$ -treated D1A cells (Fig. 4K). These data were further confirmed using promoter assay and chromatin immunoprecipitation assays (Fig. 4I–M). The results clearly demonstrated that PGI $_2$ and A β have antagonistic effects on NF- κ B transcriptional activation. Therefore, it is possible that the production of A β_{1-42} by PGI $_2$ might not be sufficient to reverse the effects of PGI $_2$ on inhibiting the NF- κ B nuclear translocation and the expression of IFN γ . PGI $_2$ does not always regulate IFN γ expression *via* A β_{1-42} .

A β_{1-42} oligomers stimulate the expression of IFN γ in APP/PS1 mouse brain. Since A β involved in the roles of PGE $_2$ and PGI $_2$ in regulating the expression of IFN γ , we continued to determine the effects of different aggregated forms of A β on the expression of IFN γ in mice. As a first step, we determined the presence of aggregated forms of A β in CSF with thioflavin T staining. The results demonstrated that A β oligomers exist in the CSF of 6-months-old APP/PS1 mice (data not shown). This observation indicated that A β oligomers in CSF might be critical for IFN γ induction. To further validate this hypothesis, we injected CSF of the APP/PS1 at 6 months of age into WT mice in the absence or presence of A β antibody (1 μ g/5 μ l). After two weeks, the mice were sacrificed and determined the expression of IFN γ . Our data revealed that APP/PS1 CSF injection (i.c.v) elevated the expression of IFN γ , which was then blocked by the A β antibody (Fig. 5A,B). Similar results were also obtained in the APP/PS1 CSF-treated D1A cells (Fig. 5C,D). This observation indicates a critical role for the A β oligomers in CSF of APP/PS1 mice in the upregulation of IFN γ expression. To more clearly understand this mechanism, we injected A β oligomers (i.c.v) into the ventricles of WT mice. The results demonstrated that IFN γ expression was upregulated (Fig. 5E,G,H). In addition, A β oligomers (1 μ M) treatment increased the expression of IFN γ in cultured slices (Fig. 5F). In agreement with these *in vivo* observations, A β oligomer treatment also induced the expression of IFN γ in D1A cells (Fig. 5I,J). More interestingly, we further found that A β mAbs blocked the effects of APP/PS1 mice CSF on stimulating NF- κ B nucleus translocation and transcriptional activity by promoter and ChIP assays in D1A cells (Fig. 5K–M). Moreover, A β oligomers were further identified as critical molecules for NF- κ B nucleus translocation and transcriptional activity (Fig. 5N–P). Collectively, our data clearly revealed the critical roles of A β oligomers in CSF of APP/PS1 mice in upregulating the expression of IFN γ .

A β_{1-42} aggregation in plaques is critical for upregulating the expression of IFN γ in APP/PS1 mice. Because IFN γ was progressively upregulated during the course of AD development, we sought to understand the role of A β fibrils or APs in upregulating the expression of IFN γ . Therefore, we first found that IFN γ was stimulated around the APs either in AD patients or 9-months-old APP/PS1 transgenic mice (Fig. 6A,B). This observation clearly indicates that APs or advanced aggregates of A β_{1-42} have the ability to stimulate the expression of IFN γ by activating astrocytes. To further explore the role of the advanced aggregate form of A β_{1-42} in IFN γ regulation, we sliced fresh brain specimens from WT mice (400 μ m) for culturing. The results demonstrated that IFN γ was activated by A β_{1-42} fibrils after 24 h of treatment (Fig. 6C). Similar results were obtained in D1A cells (Fig. 6E). In addition, the activities of astrocytes were stimulated by A β fibrils treatment (Fig. 6D). More importantly, the activities of astrocytes were progressively upregulated in AD patients (Fig. 6I). To further elucidate this mechanism, we conducted experiments to determine the effects of A β fibrils on NF- κ B transcriptional activity. The results demonstrated that A β fibrils stimulate the activity of the IFN γ promoter by activating NF- κ B in D1A cells (Fig. 6F–G). Therefore, our data revealed that not only A β_{1-42} oligomers but also A β_{1-42} fibrils have the ability to stimulate IFN γ expression by activating astrocytes, which produce high levels of IFN γ during the course of AD development.

IFN γ overproduction accelerates the progression of AD development. As the mechanisms of IFN γ induction during the course of AD development in APP/PS1 mice had been elucidated, we are prompted to investigate the roles of IFN γ in A β deposition. To achieve brain drug delivery, human IFN γ was intranasal

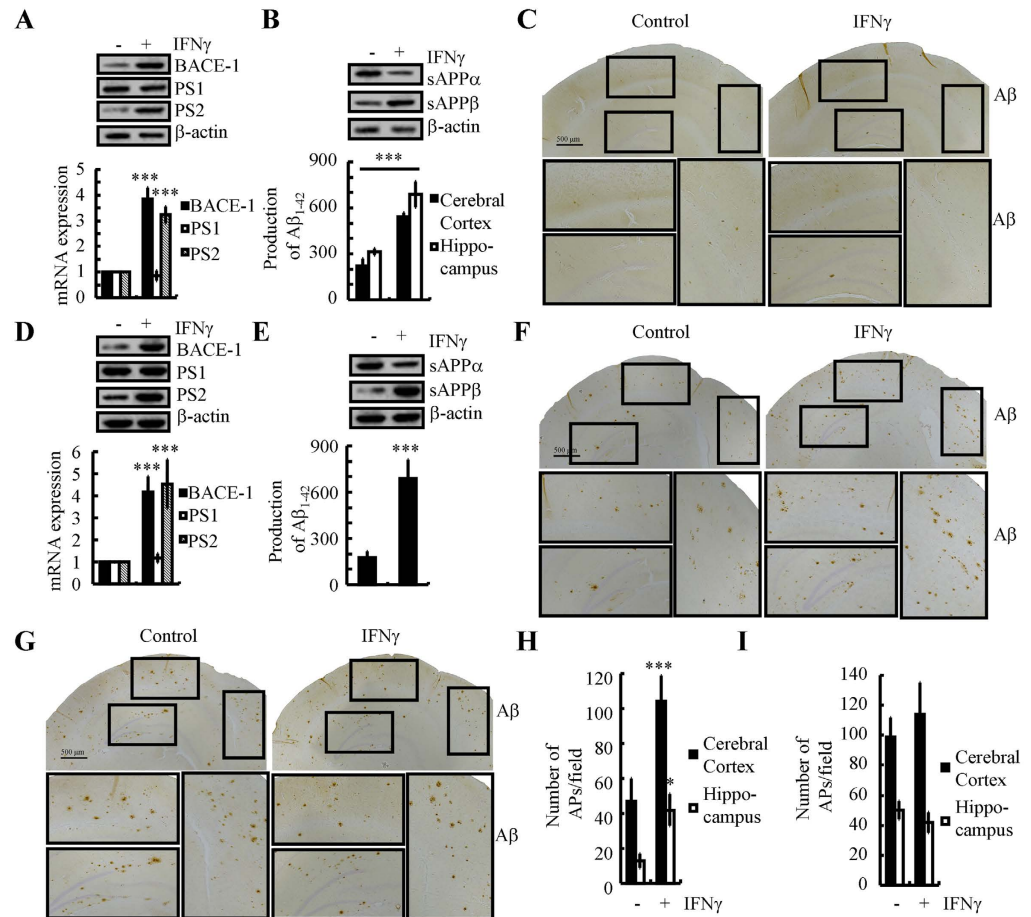


Figure 7. Nasal administration of IFN γ accelerates A β deposition in the brains of APP/PS1 mice by inducing the expression of BACE-1 and PS2. IFN γ (10 ng/20 μ l/d) was nasally administered to 3-months-old WT mice for 7 days (A–C). In select experiments, n2a cells were treated with IFN γ (10 ng/ml) for 24 h before extracting total mRNA and protein (D,E). In separate experiments, 3-months-old APP/PS1 mice was nasally administered with IFN γ (10 ng/20 μ l/d) for 3 or 6 months before determining the A β deposition in APs (F–I). The protein and mRNA expression of BACE-1, PS1 and PS2 were determined by western blot and qRT-PCR (A,D). Total amounts of β -actin and GAPDH served as an internal control. The production of sAPP α , sAPP β and A β_{1-42} was determined by western blot and A β_{1-42} enzyme immunoassay kits (B,E). Total amounts of β -actin and protein served as an internal control. The immunoreactivity of A β was determined by an immunohistochemistry assay (C,F,G). APs/field in cerebral cortex and hippocampus of APP/PS1 mice were analyzed by counting the number of APs in the images of immunohistochemistry assay (H,I). The data represent the means \pm S.E. of at least three independent experiments. * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ with respect to vehicle-treated controls.

administered to the APP/PS1 mice. After 24 h, the brains were collected and sliced by cryostats. To determine if IFN γ achieve the brains of APP/PS1 mice, the slices were stained with antibody specific reactive with human IFN γ . The results demonstrated that IFN γ not only presented in the interstitial fluid but also on the neuronal cells (data not shown). The results demonstrated that intranasally administered IFN γ (10 ng/20 μ l/d) for 7 days clearly increased the expression of BACE-1 and PS2, which resulted in accelerating the β -cleavage of APP and the production of A β_{1-42} (Fig. 7A–C). This *in vivo* observation was further verified in n2a cells (Fig. 7D,E). To further explore its roles in A β aggregation, we further treated APP/PS1 mice at the age of 3-months-old for 3 months or 6 months. The results demonstrated that A β deposition in APs is clearly elevated after 3-months-treatment, but not 6-months-treatment (Fig. 7F–I). Of note, we didn't treat WT mice with IFN γ since the production of A β_{1-42} from WT mice might not have ability to aggregate or propagate. These observations clearly demonstrated that IFN γ overproduction accelerate the production and aggregation of A β_{1-42} in APs, which exacerbate the development of AD.

When considered together, our data revealed that PGE $_2$ stimulates the synthesis of IFN γ via A β -dependent NF- κ B activation pathways. Additionally, PGI $_2$ attenuated the effects of PGE $_2$ on stimulating the expression of IFN γ by decreasing the nuclear translocation of NF- κ B. Although PGI $_2$ has the ability to upregulate the production of A β_{1-42} , the induced A β_{1-42} could not reverse the inhibitory effects of PGI $_2$ on IFN γ expression. In line with

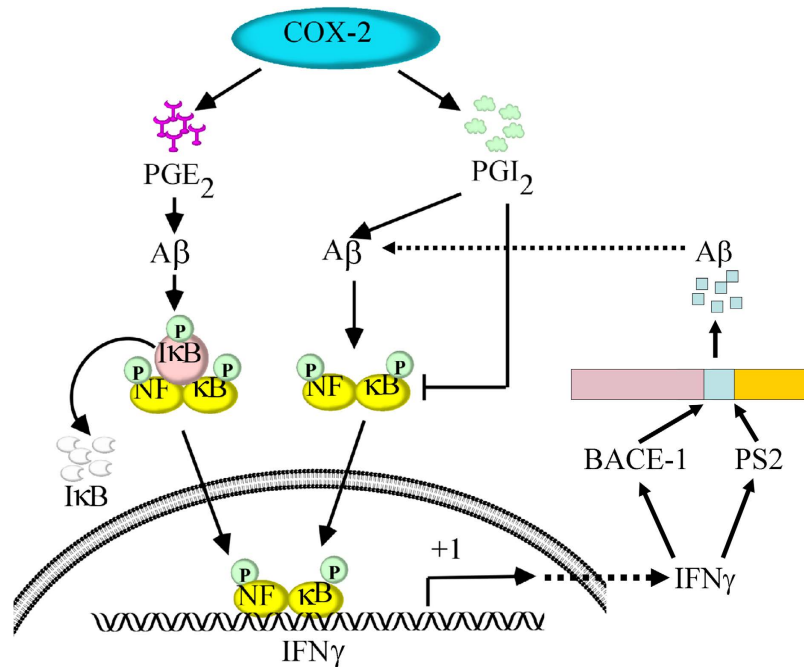


Figure 8. Signaling cascade of IFN γ upregulation during the course of AD development . COX-2 metabolic products including PGE₂ and PGI₂ displayed opposing effects on regulating the expression of IFN γ *in vitro* and *in vivo*. Specifically, PGE₂ upregulated the expression of IFN γ via an A β -dependent NF- κ B activating pathways. Although PGI₂ can stimulate the expression of IFN γ via an A β -dependent NF- κ B mechanism, PGI₂ predominantly suppresses the expression of IFN γ via NF- κ B-deactivating pathways, which is independent of A β ₁₋₄₂. Due to the role of PGE₂ and PGI₂ in inducing the production of A β ₁₋₄₂, we further found that A β ₁₋₄₂ oligomers stimulated the expression of IFN γ during the early stage of AD and that A β ₁₋₄₂ fibrils upregulated the expression of IFN γ at the late stage of AD. Highly expressed IFN γ accelerates the aggregation of A β ₁₋₄₂ in APs by inducing the expression of BACE-1 and PS2. These findings are instrumental for understanding the mechanisms of IFN γ upregulation in APP/PS1 transgenic mice and the roles of IFN γ in A β ₁₋₄₂ deposition in APs during the course of AD progression.

these *in vitro* and *in vivo* observations, IFN γ was further found to be responsible for accelerating the production and deposition of A β ₁₋₄₂. More importantly, both A β ₁₋₄₂ oligomers and A β ₁₋₄₂ fibrils have the ability to stimulate the expression of IFN γ , which potentially aggravate the pathogenesis of AD by accelerating the A β deposition in APs (Fig. 8).

Discussion

Prior work has revealed an early induction of COX-2 and of its metabolic products during the course of AD development³⁷. Therefore, we studied the role of COX-2 and its metabolic products in AD. As a powerful inducer of inflammation, COX-2 has been shown to affect the expression of IFN γ *via* its metabolic products¹³. So, we investigated the role of PGE₂ and PGI₂ in regulating the expression of IFN γ during the course of AD development. Specifically, PGE₂ stimulates the expression of IFN γ *via* A β ₁₋₄₂-dependent NF- κ B activating pathways. In contrast, PGI₂ attenuates the effects of PGE₂ on inducing the expression of IFN γ in an NF- κ B transactivating mechanism. Although A β ₁₋₄₂ reliably induces the expression of IFN γ by activating NF- κ B, PGI₂-induced A β ₁₋₄₂ might not be sufficient to reverse the inhibitory effects of PGI₂. In agreement with these *in vitro* observations, we found that PGE₂ and PGI₂ antagonistically regulated the expression of IFN γ in an A β ₁₋₄₂-dependent manner. Moreover, both A β ₁₋₄₂ oligomers and A β fibrils have the ability to upregulate the expression of IFN γ , which results in constitutively high levels of IFN γ during the course of AD development.

IFN γ is tightly regulated under physiological conditions. Although the mechanisms of IFN γ upregulation and the role of IFN γ in AD are not fully understood, it has been demonstrated that IFN γ is present or significantly elevated in the AD brain³⁸ and that IFN γ may be necessary for AD pathogenesis^{18-22,39}. In line with these observations, we found that when compared with A β deposition at 6 months of age, IFN γ was highly induced in APP/PS1 mice at 6 months of age (and occurs earlier in the cerebral cortex). In agreement with our data, Abbas *et al.*³⁸ reported that high levels of IFN γ production appeared early in the cerebral cortex (at 9 months) when compared to APs (generally at 11 months) in Tg2576 mice. Of note, this observation was supported by a series of investigations that demonstrated that IFN γ levels are increased in APP transgenic mouse brain^{40,41}. Additionally, many IFN γ -responsive genes are upregulated in AD brain^{38,42,43}. Unfortunately, this study was unable to determine how IFN γ was upregulated in the early stage of AD. For this reason, we extended the prior works to reveal the

role of A β oligomers in IFN γ induction at the early stage of AD. At the late stage of AD, A β fibrils are responsible for IFN γ stimulation, which sustained constitutively high levels of IFN γ during the course of AD development.

However, we cannot conclude that IFN γ is temporarily stimulated at the early stage of AD. As expected, we found that APs have the ability to stimulate the expression of IFN γ by activating astrocytes in APP/PS1 transgenic mice at 9 months of age. In line with our observations, it is reported that increased IFN γ production occurs in the cerebral cortex of 17–19-month-old Tg2576 mice. Here, it was observed that active astrocytes surround the β -amyloid deposits³⁸. According to this report, the highly aggregated form of A β_{1-42} might be critical for IFN γ elevation. Our results show that A β_{1-42} fibril treatment increased the expression of IFN γ expression by activating astrocytes. Yet, IFN γ is not only a passive molecule, as IFN γ has been suggested to regulate the pathogenesis of AD¹⁸. As a multiple immunoregulatory cytokine, IFN γ usually promotes the expression of other proinflammatory cytokines including TNF- α and IL-1, whose expression synergistically amplifies the effects of IFN γ on the production of A β_{1-42} . In line with this hypothesis, Blasko *et al.*³⁹ reported that costimulating human astrocytes with IFN γ , IL-1 β and TNF- α increases the synthesis of A β_{1-42} and A β_{1-40} . Therefore, the sensitive induction of A β_{1-42} following co-treatment of astrocytes with IFN γ and TNF- α is due to the upregulation of BACE-1^{21,44}. In line with these observations, we further found that IFN γ administration has ability to enhance the A β_{1-42} production by increasing the expression of BACE-1 and PS2. Interestingly, our data further revealed that IFN γ accelerated the aggregation of A β_{1-42} , but not affect the number of APs at the late stage of AD.

To keep the discussion focused, we will continue to elucidate the mechanisms of IFN γ upregulation in APP/PS1 transgenic mice. Due to the possible involvement of COX-2 metabolic products in A β deposition^{45–47}, it is easier to speculate that COX-2 signaling might be critical for IFN γ upregulation via acceleration of A β deposition. In agreement with this hypothesis, prior work has shown that NSAID treatment decreases the production of A β_{1-42} in mice⁴⁸. Specifically, celecoxib and rofecoxib treatment decreases the deposition of A β_{1-42} in AD patients and mouse models^{49,50}. The ratio of A β_{1-42} and A β_{1-40} was also elevated in COX-2/APP/PS1 mice³⁴. An *in vitro* assay revealed that PGH₂ has the ability to induce the production of A β_{1-42} ⁵¹. In addition, PGE₂ treatment increases the production of A β_{1-42} either in primary cultured mouse microglia⁵² or in C57BL/6 mice⁵³. Given the critical roles of PGE₂ in A β_{1-42} production and deposition, we predict a possible role for PGE₂ in IFN γ upregulation. As a consequence, our results demonstrate that PGE₂ treatment increases the expression of IFN γ in either astrocytes or in C57BL/6 mice. In agreement with these observations, PGE₂ treatment increases the expression of IFN γ in primary cultured rat astrocytes¹³. Along these lines, A β_{1-42} deposition might be critical for the roles of PGE₂ in upregulating the expression of IFN γ .

Interestingly, in contrast to PGE₂, PGI₂ shows suppressive effects on the expression of IFN γ . In agreement with these observations, Strassheim *et al.*¹⁷ reported that PGI₂ inhibits interferon γ (IFN γ)-stimulated cytokine expression in human monocytes. Although we could not find other evidence that suggests that PGI₂ has the ability to regulate the expression of IFN γ , it has been shown to inhibit neuroinflammation. For example, treatment with PGI₂ analogs, including iloprost and treprostinil, suppressed TNF- α expression in human myeloid dendritic cells⁹. More closely, Wahlstrom *et al.*¹⁵ reported that the administration of the PGI₂ analogue epoprostenol significantly decreased C-reactive protein (CRP) and generally decreased IL-6 levels in patients with severe traumatic brain injury compared to placebo. Schuh *et al.*¹⁶ also reported that the early induction of PGI₂ at the site of traumatic injury resulted in the aggregation of IL-1 β -expressing macrophages as a critical reason for neuropathic pain.

Because PGE₂ and PGI₂ show antagonistic effects on the expression of IFN γ , it is possible that PGE₂ and PGI₂ have the ability to regulate the activity of astrocytes or microglia. To this end, we further found that PGE₂ stimulates the activity of astrocytes by inducing the expression of GFAP. Although there is no direct evidence that supports our data, PGE₂ treatment stimulates the activity of cultured astrocytes by elevating the levels of GFAP⁵⁴. In contrast, PGI₂ suppresses the activity of astrocytes by reducing the expression of GFAP^{55,56}. The trends of astrocytes activity were similar to that of IFN γ expression. These observations not only indicated that IFN γ was produced from astrocytes, but also implied that the expression of IFN γ stimulates the activity of astrocytes. Additionally, Tsuda *et al.*⁵⁷ reported that IFN γ signaling mediates spinal microglia activation, which is responsible for neuropathic pain. In contrast to microglia activation, IFN γ shows a modest induction of GFAP⁵⁸. Given the important role of IFN γ in activating microglia and astrocytes, the receptors involved in IFN γ signaling are important. Hashioka *et al.*⁵⁹ reported that almost all IFN γ -receptor-positive cells corresponded to GFAP-positive astrocytes, whereas none of the IFN γ -receptor cells corresponded to Iba1-positive microglia cells *in vivo*. In contrast to the *in vivo* results, almost all IFN γ -receptor cells were Iba1- and GFAP-positive in cultured microglia cells⁵⁹.

Due to these observations, we next studied the involvement of NF- κ B activity in regulating the expression of IFN γ . In line with the current study, our prior work has shown that PGE₂ stimulates the expression of IL-1 β by activating the NF- κ B p65 subunit in glia¹⁰. In contrast, Raychaudhuri *et al.*⁶⁰ reported that the PGI₂ analogue treprostinil blocks NF- κ B nuclear translocation in human alveolar macrophages. These observations are in agreement with our data, which suggests that PGE₂ and PGI₂ antagonistically regulate the activity of NF- κ B. As PGE₂ and PGI₂ have the ability to induce the production of A β_{1-42} , we demonstrated that A β_{1-42} stimulates NF- κ B activity. A β_{1-42} has been previously reported to activate NF- κ B activity in neuroblastoma SH-SY5Y cells⁶¹. Due to the important role of NF- κ B in activating the IFN γ promoter⁶², we further found that NF- κ B is important for the regulation of IFN γ expression in D1a cells.

In conclusion, this study provides new evidence for the antagonistic roles of PGE₂ and PGI₂ in regulating the expression of IFN γ *in vitro* and *in vivo*. Specifically, PGE₂ upregulates the expression of IFN γ via an A β -dependent NF- κ B activating pathway. In contrast, PGI₂ attenuated the effects of PGE₂ on stimulating the expression of IFN γ . As PGI₂ displays only a modest induction of A β_{1-42} , A β_{1-42} induction was insufficient to alleviate the cells from IFN γ inhibition by PGI₂ in an NF- κ B-dependent manner. These findings provide new insights into the mechanisms of IFN γ regulation in the brain during the course of AD development.

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Author Contributions

P.W. and P.P.G. conceived and performed all of the experiments, participated in the design of the study and wrote the manuscript. X.Y., L.C.Z. and Y.N.S. carried out select experiments. P.W. (along with Z.Y.W.) interpreted the data and wrote the manuscript.

Additional Information

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