Interferon- γ Differentially Affects Alzheimer's Disease Pathologies and Induces Neurogenesis in Triple Transgenic-AD Mice

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Inflammatory processes, including the episodic and/or chronic elaboration of cytokines, have been identified as playing key roles in a number of neurological disorders. Whether these activities impart a disease-resolving and/or contributory outcome depends at least in part on the disease context, stage of pathogenesis, and cellular milieu in which these factors are released. Interferon- γ (IFN γ) is one such cytokine that produces pleiotropic effects in the brain. It is protective by ensuring maintenance of virus latency after infection, yet deleterious by recruiting and activating microglia that secrete potentially damaging factors at sites of brain injury. Using the triple-transgenic mouse model of Alzheimer's disease (3 $\times$ Tg-AD), which develops amyloid and tau pathologies in a pattern reminiscent of human Alzheimer's disease, we initiated chronic intrahippocampal expression of IFN γ through delivery of a serotype-1 recombinant adeno-associated virus vector (rAAV1-IFN γ). Ten months of IFN γ expression led to an increase in microglial activation, steady-state levels of proinflammatory cytokine and chemokine transcripts, and severity of amyloid-related pathology. In contrast, these rAAV1-IFN γ -treated 3 $\times$ Tg-AD mice also exhibited diminished phospho-tau pathology and evidence of increased neurogenesis. Overall, IFN γ mediates what seem to be diametrically opposed functions in the setting of AD-related neurodegeneration. Gaining an understanding as to how these apparently divergent functions are interrelated and controlled could elucidate new therapeutic strategies designed to harness the neuroprotective activity of IFN γ . (*Am J Pathol* 2009, 175:2076–2088; DOI: 10.2353/ajpath.2009.090059)

Dysregulated inflammatory processes have emerged as clear contributors to the progression of many neurodegenerative diseases affecting the aged, including Alzheimer's disease (AD). Environmentally derived insults combined with underlying genetic susceptibilities seem to incite inflammatory reactions, which promote the elaboration of proinflammatory cytokines, including interferon- γ (IFN γ), tumor necrosis factor- α (TNF- α), interleukin (IL) 1 β , and IL-6. This complex neuroimmune interaction, when chronically active and insufficiently controlled, ultimately leads to alterations in neural transmission, behavior, and disease-related pathogenesis (reviewed in Ref.¹).

IFN γ , a potent molecule normally expressed by natural killer cells, T cells, glia, and neurons, exhibits a multitude of immunoregulatory functions within the central nervous system (CNS) compartment, including activation of macrophages/microglia and the stimulation of macrophages to release toxic oxygen radicals (reviewed in Ref.²). The expression of IFN γ and target CD8⁺ T cells is enhanced as a function of age and neurodegeneration.^{3,4} Moreover, IFN γ enhances vascular permeability to T cells and natural killer cells and promotes the expression of TNF- α and IL-1 β by microglia (reviewed in Ref.⁵), suggesting that IFN γ is a key player in the elaboration of immune and inflammatory responses within the CNS and is involved in neurodegeneration.

Human studies have linked dysregulated IFN γ expression to neurodegenerative processes associated with AD. Blasko et al.⁶ demonstrated a correlation between IFN γ and the induction of both the amyloid plaque-associated fibrillogenic peptides, amyloid- β 1-40 (A β ₁₋₄₀) and 1-42 (A β ₁₋₄₂), and Meda et al.⁷ reported that IFN γ enhances the production of TNF- α by microglia once stimulated with A β peptides *in vitro*.⁷ Using immunocyto-

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chemical and *in situ* hybridization analyses, Abbas et al⁸ demonstrated that IFN γ expression is significantly augmented in amyloidogenic Tg2576 mice at 3 months of age (pre-amyloid pathology) and levels of this cytokine progressively increased as a function of age compared with those in wild-type mice.⁸ Interestingly, the AD mouse cohort exhibited a concomitant decrease in the anti-inflammatory cytokine IL-4, whereas an opposite cytokine expression profile was observed in the brains of wild-type mice. Others have shown that there exists a synergistic AD pathology-aggravating effect when IFN γ and TNF- α are coexpressed in the brain, leading to exacerbated A β peptide production and reduced pathogenic peptide clearance in a mouse model of AD.⁹

Based on these and other observations, we sought to further dissect the role of IFN γ in AD pathophysiology in a complex AD mouse model. Triple transgenic mice (3 $\times$ Tg-AD), which express human presenilin-1 mutant M146V (PS1^{M146V}), the Swedish mutation of human amyloid precursor protein (APP^{sw}), and the P301L mutation of human tau (tau^{P301L}), develop both plaques and neurofibrillary tangles in a progressive and age-dependent pattern.¹⁰ In this model, intracellular A β and soluble A β oligomers are first observed at 3 to 5 months of age, extracellular plaque formations appear at approximately 15 to 18 months, and neurofibrillary tangles arise at ages greater than 18 months.¹¹ These mice also exhibit early deficits in synaptic function, including long-term potentiation, which are manifested in an age-dependent manner. To assess the pathological impact of IFN γ on the patterns and severity of AD-related pathologies in 3 $\times$ Tg-AD mice but avoid developmental compensation effects that can arise in use of traditional transgenic approaches, we created a recombinant adeno-associated virus (rAAV) vector that could constitutively express human IFN γ within the CNS of adult mice after stereotactic delivery. Chronic overexpression of IFN γ by a serotype-1 recombinant rAAV vector in the 3 $\times$ Tg-AD mouse over a 10-month period revealed both potential positive and negative activities of this cytokine. Accompanying increases in proinflammatory marker transcript levels was a concomitant enhancement in the number of microglia/macrophages and intracellular A β 42 accumulation in the hippocampi of rAAV1-IFN γ -injected 3 $\times$ Tg-AD mice. Surprisingly, however, we also observed that rAAV1-IFN γ -treated 3 $\times$ Tg-AD mice exhibited evidence of reduced phospho-tau levels, enhanced synaptic marker transcript expression, and neurogenesis, suggesting that IFN γ serves seemingly juxtaposed roles in the setting of the diseased CNS.

Materials and Methods

Generation of IFN γ -Expressing rAAV Vector

The pFBGR plasmid, herein referred to as pAAV-eGFP, harbors a cytomegalovirus promoter-driven enhanced green fluorescent protein (eGFP) gene flanked by inverted terminal repeats (kindly provided by Dr. Robert Kotin). Human IFN γ cDNA from pHIF-SV- γ 1 (American

Type Culture Collection, Manassas, VA) was cloned into the pBSFBRmcs shuttle vector and subsequently into a modified pFBGR plasmid backbone devoid of the eGFP gene. The resultant plasmid was designated pAAV-IFN γ . The pAAV-IFN γ plasmid or a β -galactosidase reporter gene-expressing plasmid (pHSVlac) was transiently transfected into baby hamster kidney cells, and human IFN γ transgene expression was subsequently confirmed in cell lysates and culture supernatants by enzyme-linked immunosorbent assay (ELISA) (eBioscience, San Diego, CA) before viral packaging.

rAAV Serotype-1 Vector Packaging

The pAAV-eGFP and pAAV-IFN γ plasmids were separately transposed into DH10-BAC *Escherichia coli* (Invitrogen, Carlsbad, CA), and DNA was purified before transfection into SF9 cells to produce baculovirus. Plasmids encoding AAV Rep and serotype 1 Cap genes were separately transposed into DH10-BAC cells. Resultant baculovirus stocks harboring pAAV-eGFP or pAAV-IFN γ were co-introduced into SF9 insect cells with baculovirus preparations expressing Rep and Cap to produce serotype 1 rAAV vectors according to methodology published previously.¹² Recombinant AAV1-IFN γ and AAV1-eGFP virions were purified over a cesium chloride gradient and collected at a refractive index of 1.374 to 1.370. 293A cells were transduced with either rAAV1-IFN γ or rAAV1-eGFP to obtain titer information via flow cytometry and quantitative real-time PCR, and titers were expressed as transducing units per ml.¹³

Transgenic Mice

The 3 $\times$ Tg-AD mice were kindly provided by Frank LaFerla (University of California, Irvine, Irvine, CA). All animal were housed and procedures were performed in compliance with guidelines established by the University Committee of Animal Resources at the University of Rochester.

Stereotactic Infusion of rAAV1-IFN γ and rAAV1-eGFP

Two-month-old 3 $\times$ Tg-AD mice ($N = 4$ to 5 per experimental group) were stereotactically injected intrahippocampally with either rAAV1-IFN γ or rAAV1-eGFP in accordance with approved University of Rochester animal use guidelines. Mice were anesthetized with Avertin (300 mg/kg) and monitored throughout the stereotactic procedure for maintenance of anesthesia. The mouse was positioned in a stereotactic apparatus (ASI Instruments, Warren, MI), the skull was exposed via a sagittal incision, and two burr holes were drilled bilaterally over the designated hippocampal coordinates: (i) bregma -2.7 mm, lateral 2.0 mm, and ventral 1.3 mm; and (ii) bregma -2.9 mm, lateral 2.5 mm, and ventral 1.7 mm. A 33-gauge needle was gradually advanced to the desired depth over a 1.5-minute period. All injections were per-

formed using a microprocessor controlled pump (Ultra-Micro-Pump, WPI Instruments, Sarasota, FL). A total of 2 μ l (3×10^9 transducing units) per injection was delivered at a constant rate of 200 nl/min. After each injection, the needle was extracted over a 3-minute period. After the final injection, the incision was closed using a Vicryl suture, a topical 5% lidocaine ointment (Fougera, Melville, NY) was applied, and the mice were allowed to recuperate in a heated recovery chamber before returning to their housing cage and the vivarium. Most mice were housed until 12 months of age, at which time they were sacrificed via pentobarbital overdose and transcardiac perfusion with saline (0.9% sodium chloride).¹⁴ The brains were removed and bisected with the right hemisphere being submerged in 4% paraformaldehyde in 0.1 mol/L phosphate buffer (PB) and the left hemisphere being microdissected and frozen at -80°C for subsequent RNA isolation. A subset of mice received daily i.p. injections of 5-bromo-2'-deoxyuridine (BrdU) (50 mg/kg, Sigma-Aldrich, St. Louis, MO) for 3 days at 35 days after rAAV vector injection and were sacrificed 2 days after the final BrdU injection as described above.

IFN γ ELISA

Mouse hippocampal tissue was harvested and snap-frozen on dry ice followed by storage at 80°C . Frozen tissue was homogenized using a motorized pestle in 10 μ l of lysis solution per milligram of tissue. Lysis solution consisted of 10 mmol/L Tris, pH 8.0, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride prepared in ethanol added just before use, 1% Triton X-100, and 5 μ l/ml protease inhibitor (all chemicals obtained from Sigma-Aldrich). Tissue was incubated in lysis solution for 1 hour at 4°C , followed by centrifugation at $10,000 \times g$ for 10 minutes to remove cellular debris. The supernatant was removed and diluted 1:10 or 1:50 for analysis of total protein content and for IFN γ concentrations by capture ELISA (R&D Systems, Minneapolis, MN). The assay was performed according to the manufacturer's instructions. In brief, capture antibody was reconstituted and diluted to 4 mg/ml, and 100 μ l was added to each well of 96-well microplate flat-bottom plates (Imm lux HB Dynex Technologies, Chantilly, VA) and incubated overnight at 27°C . Plates were washed three times with wash buffer (PBS + 0.05% Tween 20), followed by blocking for 1 hour at 27°C with PBS + 0.05% Tween 20 + 1% bovine serum albumin and 0.05% NaN_3 . Standards and samples were diluted with Reagent Diluent (DY995, R&D Systems) and diluted 1:100 with Tris-buffered saline + 0.05% Tween 20, pH 7.2 to 7.4.). Plates were washed three times with wash buffer, samples were added, and the plates were incubated for 2 hours at 27°C . Detection antibody was reconstituted with 1 ml of Reagent Diluent and diluted to 50 ng/ml. Plates were washed three times with wash buffer, detection antibody was added to each well, and the plates were allowed to incubate for 2 hours at 27°C . Plates were washed 3 times with wash buffer, 100 μ l of streptavidin horseradish peroxidase diluted 1:200 was added, and plates were allowed to incubate

for 20 minutes at 27°C . Plates were washed three times with wash buffer and developed with tetramethylbenzidine in phosphate citrate buffer (Sigma-Aldrich) for 15 minutes at 27°C and quenched with 2 N H_2SO_4 . Absorbance readings were taken at 450 nm using a plate reader (BioRad, Hercules, CA).

Antibodies

The following antibodies were used at the designated working dilutions: anti-amyloid precursor protein A4, amino acids 66 to 81 corresponding to the NPXY motif of human APP (Clone Y188, 1:750, Abcam, Cambridge, MA); anti-amyloid β 1-42 clone 12F4 reactive to the C terminus of β -amyloid and specific for the isoform ending at amino acid 42 (1:1000, Covance, Berkeley, CA); anti-amyloid β 1-42 polyclonal antibody for intracellular amyloid- β staining (1:1000, Invitrogen [formerly Biosource, Hopkinton, MA]); HT7 antibody, reactive to the epitope corresponding to human tau amino acid residues 159 to 163 (1:200, Pierce, Rockford, IL); AT180 antibody, specific for human tau phosphorylated at threonine 231 and serine 235 (1:200, Pierce); CP-13 antibody, specific for human tau phosphorylated at serine 202 (1:200, kindly provided by Dr. P. Davies); anti-human anti-glial fibrillary acidic protein (GFAP) (1:1000, Dako Cytomation, Glostrup, Denmark); anti-IBA-1 (1:750, Wako Chemicals USA, Inc., Richmond, VA) for detection of mouse microglia and monocytes; anti-human IFN γ (5 μ g/ml, R&D Systems); anti-GFP (1:2000, Invitrogen, Eugene, OR); anti-NeuN (1:200, Millipore, Billerica, MA) for mature neurons; anti-doublecortin (DCX) (1:750, Abcam, Cambridge, MA) for immature neurons; and anti-BrdU (1:750, BD Pharmingen, San Jose, CA).

Immunohistochemistry

The right hemispheres from surgically manipulated mice were postfixed for 24 hours in 4% paraformaldehyde in 0.1 mol/L PB, followed by equilibration in 20% sucrose in 0.1 mol/L PBS and then 30% sucrose in 0.1 mol/L PBS. Brains were coronally sectioned on the freezing stage of a sliding microtome (Microm, Walldorf, Germany) at 30 μ m and stored in cryoprotectant at -20°C until immunohistochemical processing. Brain sections were washed with 0.15 mol/L PB for 2 hours to remove the cryoprotectant and then were incubated with 3% H_2O_2 in 0.15 mol/L PB for 20 minutes to quench endogenous peroxidase activity. For A β peptide-specific stains, the sections were treated with 70% formic acid for 15 minutes for epitope retrieval. For the intracellular A β 1-42 in place of formic acid the sections were mounted onto slides and allowed to dry. The target buffer was heated to 98°C in a microwave oven (GE, Louisville, KY), and the slides were submerged into the buffer and placed in the microwave twice for 3 minutes at 450 W, and allowed to rest for 5 minutes between microwave steps. Brain sections were processed similarly for immunohistochemical analysis as detailed below. The sections were washed and permeabilized in 0.15 mol/L PB and 0.4% Triton X-100, followed

by blocking in 0.15 mol/L PB with 10% normal goat serum and 0.4% Triton X-100. After blocking, the sections were incubated in 0.15 mol/L PB with 1% normal goat serum and 0.4% Triton X-100, with the designated primary antibody. The sections were washed with 0.15 mol/L PB, followed by an incubation with the appropriate biotin-conjugated secondary antibodies (1:1000, Vector Laboratories, Burlingame, CA) in 0.15 mol/L PB with 1% normal goat serum and 0.4% Triton X-100. The sections were washed with 0.15 mol/L PB with 1% normal goat serum and 0.4% Triton X-100, and incubated in the avidin-biotin complex per the manufacturer's protocol (Vectastain ABC System, Vector Laboratories). Sections were washed in 0.15 mol/L PB followed by rinses in distilled water. The sections were developed with nickel-enhanced diaminobenzidine (Vector Laboratories). Sections were mounted on Superfrost Plus slides (VWR International, West Chester, PA), coverslipped, and viewed using an Olympus AX-70 microscope and motorized stage (Olympus, Center Valley, PA), and the MCID 6.0 Imaging software (Interfocus Imaging/GE Healthcare, Cambridge, UK) was used for quantifying all stains.

eGFP and human IFN γ immunocytochemical analyses were performed using fluorescently labeled secondary antibodies. Tissue sections were washed with PBS to remove cyroprotectant as described above. Sections were permeabilized with PBS + 0.1% Triton X-100 for 5 minutes and then incubated with blocking serum (PBS + 0.1% Triton X-100 and 10% donkey serum) for 1 hour at 22°C. Primary antibody was diluted into PBS + 0.1% Triton X-100 + 0.1% donkey serum overnight at 4°C. Sections were washed three times for 10 minutes each and then incubated in secondary antibody for 2 hours at 22°C. Anti-goat Alexa 568 (1:500, Invitrogen) was used for human IFN γ immunohistochemistry and anti-rabbit Alexa 488 (1:500, Invitrogen) was used for eGFP immunohistochemistry. Sections were mounted and visualized using a Leica TCS SP1 confocal scanning microscope (Leica Microsystems, Bannockburn, IL) using LCS software (Leica Microsystems).

For BrdU immunohistochemistry, tissue sections were washed with PBS to remove cyroprotectant, as above, then incubated with 3% H₂O₂ in 0.15 mol/L PB for 20 minutes to quench endogenous peroxidase activity. The sections were permeabilized using 0.15 mol/L PB with 0.4% Triton X-100 (Sigma-Aldrich), followed by a denaturing step using 2 N HCl with 0.5% Triton X-100 for 30 minutes. The tissue was neutralized using 0.07 N NaOH for 2 minutes, followed by blocking in 0.15 mol/L PB + 0.4% Triton X-100 + 10% normal goat serum for 2 hours. BrdU-positive cells were detected using a mouse monoclonal anti-BrdU antibody (1:750, BD Pharmingen). The sections were washed with 0.15 mol/L PB, followed by an incubation with goat anti-mouse, biotin-conjugated secondary antibodies (1:1000, Vector Laboratories) in 0.15 mol/L PB with 1% normal goat serum and 0.4% Triton X-100. The sections were washed with 0.15 mol/L PB with 1% normal goat serum and 0.4% Triton X-100 and incubated in the avidin-biotin complex per the manufacturer's protocol (Vectastain ABC System). Sections were washed in 0.15 mol/L PB followed by rinses in distilled

water. The sections were developed with nickel-enhanced diaminobenzidine. Sections were imaged as described above.

For fluorescent co-immunocytochemical detection of NeuN and BrdU, brain sections were processed as described above with the following modifications. Tissue was initially incubated with mouse monoclonal anti-BrdU antibody overnight at 4°C. The sections were washed with 0.15 mol/L PB, followed by incubation with a goat anti-mouse Alexa 568 secondary antibody (1:1000). Sections were then washed and incubated for 4 hours with a mouse monoclonal anti-NeuN antibody conjugated with Alexa 405 (1:500, Millipore, Bedford, MA). Sections colabeled for DCX or GFAP and BrdU were permeabilized and blocked as described above. Tissue was initially incubated with mouse monoclonal anti-BrdU antibody and rabbit polyclonal anti-DCX or rabbit polyclonal anti-GFAP overnight at 4°C. The sections were washed with 0.15 mol/L PB, followed by incubation with goat anti-mouse Alexa 568 secondary antibodies and goat anti-rabbit Alexa 405 (1:1000, Invitrogen). Sections were washed, mounted, and imaged as described above.

Quantitation of Staining Intensities

Quantification of positively stained targets was done with the image analysis tools in the MCID program (Interfocus Imaging/GE Healthcare) allowing for a target intensity threshold for staining to be set, and a target pixel count to be assessed and compared with the total scanned area. In brief, $\times 20$ images in the CA₁ hippocampal regions, corresponding to 2.5 to 3.0 mm posterior from bregma, the area encompassing the sites of injection, were captured along the length of the pyramidal layer of the CA1 using an Olympus AX-70 microscope equipped with a motorized stage (Olympus, Melville, NY). All images were digitally analyzed based on a target threshold for pixel intensity. Fifteen $\times 20$ images from five hippocampal sections per rAAV vector-injected mouse were analyzed. Counts were averaged across all images and mice within a group, and all statistics were analyzed using Prism GraphPad software (GraphPad Software, San Diego CA).

Quantitative Real-Time RT-PCR Analysis

RNA was isolated from microdissected hippocampi of rAAV1-IFN γ - or rAAV1-eGFP-injected 3 $\times$ Tg-AD mice with TRIzol solution (Invitrogen), as described previously.¹⁵ RNA was treated with RQ1 DNase (Promega, Madison, WI) to selectively degrade any contaminating genomic DNA, followed by phenol-chloroform extraction and ethanol precipitation. One microgram of total RNA was reverse transcribed using a High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). An aliquot of cDNA (100 ng) was used to assess the levels of 17 targets per mouse and was analyzed in a standard PE7900HT quantitative RT-PCR reaction using TaqMan Assay-on-Demand primer probe sets in Microfluidic cards (Applied Biosystems) and 100 μ l of MasterMix containing HotStart DNA polymerase (Eurogentec, Seraing,

Belgium). 18S RNA served as the control to which all samples were normalized (Applied Biosystems). The resultant data were analyzed using the $\Delta\Delta C_T$ method, normalizing the rAAV1-IFN γ values to samples from rAAV1-eGFP-injected 3 $\times$ Tg-AD mice. To determine statistical significance, Student's *t*-test was used.

Results

Creation and Initial Testing of an IFN γ -Expressing Recombinant Adeno-Associated Virus Vector

To assess the potential effects that chronic expression of IFN γ may exact on the progression and severity of AD-related pathologies in 3 $\times$ Tg-AD mice, two rAAV vectors were constructed: one expressing human IFN γ (rAAV1-IFN γ) and a second expressing the reporter gene product, enhanced green fluorescent protein (rAAV1-eGFP). The human homolog of IFN γ was used in this study to facilitate immunological differentiation of the rAAV-vectored transgene from endogenous mouse IFN γ . The individual transgenes were placed under the transcriptional control of the human cytomegalovirus promoter (Figure 1A). An ELISA performed on lysates and culture supernatants derived from transiently transfected baby hamster kidney cells confirmed that human IFN γ was robustly expressed from the rAAV1-IFN γ plasmid construct (Figure 1B). Both rAAV1-IFN γ and rAAV1-eGFP vectors were subsequently packaged into serotype 1 virions using a previously described baculovirus-based rAAV vector production method.¹²

The rAAV1-IFN γ and rAAV1-eGFP control vectors were stereotactically delivered to the CA1 layer of the 3 $\times$ Tg-AD at 2 months of age. At this early age, 3 $\times$ Tg-AD mice do not harbor detectable extracellular human APP/A β or hyperphosphorylated tau as determined by immunohistochemical methods.^{10,11} Mice were sacrificed 10 months after injection to assess vector-mediated expression. Respective transgene expression from both rAAV1-eGFP and rAAV1-IFN γ was detectable within the hippocampus 10 months after the stereotactic infusion (Figure 1, C–F). Vector-mediated transduction was confined to the hippocampal formation, as examination of more distal brain regions did not reveal any rAAV vector transgene-expressing cells (data not shown). Co-immunocytochemical analyses of rAAV1-eGFP injected 3 $\times$ Tg-AD mice to determine the major cell type(s) initially transduced showed that eGFP positivity was localized primarily to cells expressing the mature neuronal marker NeuN (Figure 1H), although rarely detectable within astrocytes or microglia (Figure 1, I and J, respectively). Stereotactic infusion of a separate cohort of 3 $\times$ Tg-AD mice at 9 months of age and subsequent ELISA-based analysis of microdissected tissue at 12 months of age indicated that rAAV1-IFN γ injected mice produced significantly higher levels of human IFN γ in hippocampal tissue than rAAV1-eGFP injected mice (Figure 1G).

Quantitative Real-Time RT-PCR Analysis of mRNA from 3 $\times$ Tg-AD Mice Injected with rAAV1-IFN γ and rAAV1-eGFP Revealed a Number of Differences in Proinflammatory Molecule Expression Profiles

The rAAV1-IFN γ and rAAV1-eGFP control vectors were bilaterally delivered to the CA1 layer of a new cohort of 3 $\times$ Tg-AD mice at 2 months of age ($N = 4$ to 5 per experimental group). Ten months later, the right hemisphere of each brain was postfixed in 4% paraformaldehyde, equilibrated in sucrose, and sectioned for immunohistochemical analyses. Left hemisphere tissue was microdissected to remove the vector-infused hippocampi, mRNA was isolated, and quantitative real-time RT-PCR subsequently performed for several proinflammatory mediators, whose transcript expression levels modulate in response to IFN γ . Ten months of IFN γ expression activated a broad spectrum of factors associated with proinflammatory processes, including the chemokine (C-C motif) ligand 2 (CCL2), CCL3, CCL6, TNF- α , and its cognate receptors, TNFRsf1a and TNFRsf1b, as well as transcription factors c-Jun and NF κ B2 (Table 1). In contrast to what our group has previously observed after induction of TNF- α responses in 3 $\times$ Tg-AD and nontransgenic mice,¹³ chronic expression of IFN γ led to a small but significant down-regulation of the C3 component of complement (-1.53 ± 0.1436 -fold, $P < 0.05$).

Chronic IFN γ Expression Leads to Marked Activation of Microglia but Seems to Minimally Affect Astrocyte Status

Mice infused with rAAV1-IFN γ showed a minimal, but significant, 1.175-fold enhancement of astrocyte marker GFAP transcript levels compared with 3 $\times$ Tg-AD mice injected with rAAV1-eGFP (Figure 2A). This slight increase in GFAP transcript level was not substantiated by immunohistochemical/stereological analyses of astrocyte numbers in the identically treated contralateral hemisphere, where rAAV1-IFN γ and rAAV1-eGFP injected animals exhibited no difference in numbers of GFAP-positive astrocytes (Figure 2, B–D). Analyses of steady-state transcript levels for two markers, egf-like module containing, mucin-like, hormone receptor-like 1 (Emr1; also known as F4/80) for microglia/macrophages, and integrin alpha X (Itgax; also known as CD11c) for all dendritic-like cells, including microglia, revealed interesting disparities. Although transcript levels for F4/80 remained comparable, CD11c was significantly enhanced in the hippocampi of mice receiving rAAV1-IFN γ (Figure 2A). Moreover, immunohistochemical staining for the macrophage/microglia marker IBA-1 revealed a significant enhancement in IBA-1⁺ cells within the rAAV1-IFN γ -transduced hippocampus compared with rAAV1-eGFP-infused control animals, indicating that unlike astrocytes, microglia are markedly affected by the prolonged presence of IFN γ in 3 $\times$ Tg-AD mouse brain (Figure 2, E–G).

Figure 1. Generation and initial testing of a serotype-1 rAAV vector expressing human IFN γ . **A:** The cDNA encoding human IFN γ (hIFN γ) or eGFP reporter protein was cloned into a rAAV shuttle vector under the transcriptional control of the cytomegalovirus immediate-early promoter to create pAAV-IFN γ and pAAV-eGFP, respectively. **B:** Baby hamster kidney cells were transiently transfected with either pAAV-IFN γ or pAAV-eGFP, and expression of human IFN γ in cellular lysates or supernatant samples was determined by ELISA. A nontransfection control (NTC) was used to determine assay background. Error bars indicate SD. **C-F:** pAAV-IFN γ and pAAV-eGFP were subsequently packaged into serotype-1 rAAV particles. Two-month-old 3 $\times$ Tg-AD mice ($N = 4$ to 5 per experimental group) were bilaterally infused with 2 μ l ($\sim 3 \times 10^9$ transducing particles) of rAAV1-IFN γ or 2 μ l of rAAV1-eGFP, and sacrificed 10 months later. Brains were sectioned and processed for immunocytochemistry for human IFN γ (red; **C** and **E**) or eGFP (green; **D** and **F**). Scale bar in **F** = 10 μ m. Subsets of sections from rAAV1-eGFP injected mice were processed for co-immunocytochemistry for the neuronal markers NeuN and eGFP (**H**), astrocytic marker GFAP and eGFP (**I**), and microglial marker IBA-1 and eGFP (**J**) to determine the cell type(s) transduced by the serotype-1 rAAV vector particles. Scale bar in **J** = 10 μ m. **G:** Nine-month-old 3 $\times$ Tg-AD mice ($N = 4$ per experimental group) were stereotactically infused with 2 μ l ($\sim 3 \times 10^9$ transducing particles) of rAAV1-IFN γ into the ipsilateral hippocampus and 2 μ l of rAAV1-eGFP into the contralateral hippocampus, and sacrificed 3 months later. Injection sites were microdissected, and lysates were generated and analyzed by ELISA to assess IFN γ levels. Error bars indicate SEM. * $P < 0.05$.

Intracellular A β -Related Pathology Is Significantly Exacerbated by Chronic IFN γ Expression

Quantitative RT-PCR was subsequently performed to assess the extent to which IFN γ expression influenced human APP^{swE} transgene expression and its endogenous mouse wild-type counterpart. No statistically significant changes were detected in either APP homolog when rAAV1-IFN γ mice were compared with rAAV1-eGFP control animals (Figure 3A), although a slight trending in-

crease in human APP^{swE} steady-state RNA sequences could be observed in IFN γ -expressing mice (1.449 ± 0.2396 -fold, $P = 0.0839$). This human APP^{swE} transcript level difference did not correlate to immunohistochemical analysis of human APP protein using the Y188 antibody, in that both cohorts stained to similar intensities (Figure 3, B-D). Immunostaining with the human APP (hAPP)/A β -specific monoclonal antibody 6E10 revealed that rAAV1-IFN γ -infused 3 $\times$ Tg-AD mice stain significantly more intensely than rAAV1-eGFP controls (Figure 3, E-G), suggesting that the former may harbor higher levels of

Table 1. Fold Change in Steady-State Proinflammatory Transcript Expression in 3×Tg-AD Mice Injected with rAAV1-IFN γ versus rAAV1-eGFP at 10 Months Post-Transduction

Target Gene	Fold-change rAAV1-IFN γ - versus rAAV1-eGFP-infused 3×Tg-AD mice	SEM	P values for significance (Student <i>t</i> -test)
<i>CCL2 (MCP-1)</i>	2.1399	0.3565	<0.01
<i>CCL3 (MIP-1α)</i>	1.8079	0.1982	<0.01
<i>CCL6 (MRP-1)</i>	1.8787	0.0937	<0.001
<i>TNF-α</i>	2.9663	0.6033	<0.01
<i>TNFrα1a</i>	1.4606	0.0754	<0.001
<i>TNFrα1b</i>	1.4793	0.1261	<0.01
<i>C3</i>	−1.5346	0.1436	<0.05
<i>Jun</i>	1.8071	0.0673	<0.001
<i>NFκb2</i>	2.2097	0.1907	<0.001

Transcriptional analysis of 3×Tg-AD mice injected with rAAV1-eGFP or rAAV1-IFN γ for additional proinflammatory markers of interest. Total RNA was purified from microdissected hippocampus from 12-month-old 3×Tg-AD mice that had been previously stereotactically infused with rAAV1-IFN γ or rAAV1-eGFP. cDNA pools were generated and subjected to Applied Biosystems Microfluidic Card analysis of additional inflammation-related transcriptional targets. Fold changes in steady-state transcript levels in rAAV1-IFN γ - versus rAAV1-eGFP-injected animals were calculated with SEM and subsequently tested for significance by Student's *t*-test.

intracellular A β peptides, which have been proposed to play an early triggering role in AD-related neurodegeneration.^{16–19} To further detail the evolution of intracellular A β pathology in rAAV1 vector-injected 3×Tg-AD mice, we used an immunohistochemical staining method optimized for visualization of intracellular A β 42 peptide,^{20,21} which more readily unmask intracellular A β peptide epitopes than standard formic acid epitope retrieval methods used for extracellular plaque immunohistochemistry. Using this methodology, we have been able to reproducibly detect intracellular A β 42 in 3×Tg-AD mice.¹¹ In the present study, intracellular A β 42 staining intensity was markedly enhanced in rAAV1-IFN γ injected 3×Tg-AD mice compared with control mice (Figure 3, H–J), further substantiating the hypothesis that IFN γ exacerbates AD-related pathogenic processes.

rAAV1-IFN γ -Infused 3×Tg-AD Mice Exhibit an Unexpected Reduction in Immunohistochemically Detectable Phospho-Tau Pathology

In addition to a human APP^{swE} transgene and presenilin-1 knock-in mutation, 3×Tg-AD mice harbor a human tau^{P301L} mutant transgene, which results in progressive elaboration of pathogenic hyperphosphorylated forms of tau beginning at 9 months of age and eventually neurofibrillary tangle-like pathology by 18 months.^{10,11} Immunohistochemical analysis of human tau transgene expression using the HT7 antibody and subsequent quantitation indicated that rAAV1-IFN γ transduction did not overtly affect tau expression compared with rAAV1-eGFP-injected control animals (Figure 4, A–C). We then incubated brain sections with the anti-human phosphorylated tau AT180 antibody, specific for human tau phosphorylated at the Thr²³¹Ser²³⁵ residues, or the CP-13 antibody, which is specific for human tau phosphorylated at Ser²⁰². Quantitation of AT180 and CP-13 staining intensities revealed marked reductions specifically within rAAV1-IFN γ -infused 3×Tg-AD mice, indicating that chronic IFN γ expression differentially affects amyloid- and tau-related pathologies in this mouse model (Figure 4, D–F and G–I).

IFN γ Expression Enhances Neurogenesis within the Hippocampi of rAAV1-IFN γ -Transduced 3×Tg-AD Mice

The unexpected “protective” or “pathology-suppressing” effect that chronic IFN γ expression produced on phospho-tau staining patterns within 3×Tg-AD mice led us to monitor neuronal status in these animals at 10 months after transduction. 3×Tg-AD mice that received rAAV1-IFN γ exhibited increases in mRNA expression of a number of synaptic markers. PSD-95, a specialized scaffold protein in the postsynaptic protein complex, was 1.228 ± 0.0556 -fold ($P < 0.01$) greater in rAAV1-eGFP injected 3×Tg-AD mice (Figure 5A). Dynamin-1, a GTPase expressed exclusively within the CNS, which is important in the formation and recycling of synaptic vesicles,²² was enhanced by 1.463 ± 0.077 -fold ($P < 0.01$) in the rAAV1-IFN γ cohort. The transcript encoding synaptophysin, an integral membrane protein found in synaptic vesicles of neurons,²³ was up-regulated 1.873 ± 0.0722 -fold ($P < 0.001$) in 3×Tg-AD mice injected with rAAV1-human IFN γ . In aggregate, the enhancement of these transcripts suggested that chronic IFN γ expression was promoting a process suggestive of synaptogenesis in the 3×Tg-AD brain.

To determine the IFN γ effects on markers for mature and progenitor neurons, we initially analyzed brain sections for NeuN, a marker of mature neuronal nuclei. Mice injected with rAAV1-eGFP (Figure 5B) showed qualitatively reduced staining intensity compared with the mice injected with rAAV1-IFN γ (Figure 5C). Quantitation of staining intensities across the treatment groups confirmed higher NeuN signal in rAAV1-IFN γ -injected 3×Tg-AD mice (Figure 5D). An antibody specific for DCX was used to identify immature neurons in the process of neural migration, as this marker is found in the soma of migrating neurons and the axons of differentiating neurons.²⁴ Mice injected with rAAV1-IFN γ exhibit an increase in DCX nuclear staining in cells residing in the CA1 of the hippocampus (Figure 5F) compared with mice injected with rAAV1-eGFP (Figure 5E). When quantified there was a significant increase in DCX⁺ staining intensities in mice injected with rAAV1-IFN γ for within the CA1

A Changes in Steady-state Glial Marker Transcript Levels

Figure 2. Differential activation of microglia and astrocytes after chronic IFN γ expression. Two-month-old 3 $\times$ Tg-AD mice ($N = 4$ to 5 per experimental group) were stereotactically infused bilaterally twice with 2 μ l ($\sim 3 \times 10^9$ transducing particles) of rAAV1-IFN γ or rAAV1-eGFP and sacrificed 10 months later. **A:** The left hemisphere was processed for total RNA isolation and transcriptional marker levels for astrocytes and microglia were analyzed via a PE7900HT quantitative real-time RT-PCR reaction. Relative fold changes in transcript levels for the astrocyte marker GFAP and macrophage/microglial markers F4/80 and Cd11c are reported. Error bars indicate SEM. * $P < 0.05$; *** $P < 0.001$. **B–G:** The right hemisphere was processed for immunohistochemistry to detect astrocytes (anti-GFAP; **B** and **C**) or macrophages/microglia (anti-IBA-1; **E** and **F**). Insets in **C** and **F** represent digitally magnified images of the respective outlined region in each photomicrograph for better visualization of stained cell morphology. Scale bar in **F** = 200 μ m. Quantitative image analyses for cells staining positive for GFAP (**D**) and IBA-1 (**G**) were performed on each brain and are presented in histogram format. Error bars indicate SEM. *** $P < 0.001$.

hippocampus (Figure 5G), suggesting that IFN γ imparts a positive influence on neurogenesis.

Additional subsets of mice were intrahippocampally injected with either rAAV1-eGFP or rAAV1-IFN γ , and 35 days postinjection received daily intraperitoneal injections of BrdU for 3 days and were sacrificed 2 days after the final BrdU injection. Brains were postfixed with paraformaldehyde, sectioned, and incubated with antibodies specific for BrdU alone with diaminobenzidine development to facilitate quantitation. Cells with rounded cell bodies staining robustly for BrdU, reminiscent of progenitor morphology, were subsequently quantified within the CA1 region of the hippocampus and dentate gyrus using MCID software, and post hoc statistical analyses were performed. Mice injected with rAAV1-eGFP harbored qualitatively few BrdU⁺ cells that met the established morphological criteria for migrating progenitor cells within the CA1 subfield of the hippocampus (Figure 6A). In contrast, mice injected with rAAV1-IFN γ possessed a number of rounded darkly stained bodies slightly dorsal to the pyramidal layer of the CA1 subfield (Figure 6B). The rAAV1-IFN γ -injected animals exhibited a statistically significant enhancement in BrdU⁺ cell numbers within the injected CA1, whereas the differences in numbers of BrdU⁺ cells within the more distal dentate gyrus did not quite reach significance compared with rAAV1-eGFP control mice (Figure 6C).

Brain sections from rAAV vector-injected, BrdU-pulsed mice were subsequently co-incubated with a BrdU-specific antibody and antibodies specific for various cellular

markers to determine the identity of the enhanced proliferating population in the rAAV1-IFN γ cohort. We found that BrdU⁺ cells within the CA1 subfield of mice injected with rAAV1-eGFP did not stain for NeuN, a mature neuronal marker (Figure 6D), whereas a subset of BrdU⁺ cells in mice injected with rAAV1-IFN γ did stain positive for NeuN (Figure 6E) and exhibited a quantitative increase in numbers of NeuN⁺/BrdU⁺ cells (Figure 6F). Further examination of neuroprogenitor cells using an antibody specific for DCX revealed that rAAV1-IFN γ -injected mice harbored a nearly significant increase in BrdU⁺/DCX⁺ cells compared with rAAV1-eGFP control mice (Figure 6, G–I). In addition, we assessed whether any BrdU⁺ cell subsets expressed GFAP, a marker for astrocytes, or the microglial marker IBA-1. Rarely did we observe colocalization of BrdU and GFAP or BrdU and IBA-1 signals in either rAAV1-eGFP controls (Figure 6, J and L, respectively) or rAAV1-IFN γ -injected mice (Figure 6, K and M, respectively), indicating that a majority of the proliferating population induced by chronic IFN γ expression belonged to the neuronal lineage.

Discussion

For more than a decade, it was widely accepted that microglia were the sole cell type responsible for expressing IFN γ within the brain, but evidence has since revealed that many CNS-resident cells, including neurons

Figure 3. Chronic IFN γ expression enhances APP/A β -related pathology. Two-month-old 3 $\times$ Tg-AD mice ($N = 4$ to 5 per experimental group) were stereotactically infused bilaterally twice with 2 μ l ($\sim 3 \times 10^9$ transducing particles) of rAAV1-IFN γ or rAAV1-eGFP and sacrificed 10 months later. **A:** The left hemisphere was processed for total RNA isolation and transcriptional marker levels for human and mouse APP were analyzed via a PE7900HT quantitative real-time RT-PCR reaction. Relative fold changes in transcript levels are reported. Error bars indicate SEM. **B–J:** The right hemisphere was processed for immunohistochemistry to detect human APP^{swE} transgene product (Y188; **B** and **C**), human APP/A β (6E10; **E** and **F**), or intracellular A β _{1–42} (anti-A β 42; **H** and **I**). **Insets** in **C**, **F**, and **I** represent digitally magnified images of the respective outlined region in each photomicrograph for better visualization of stained cell morphology. Scale bar: 200 μ m (**E**); 100 μ m (**H**). Quantitative image analyses for cells staining positive for hAPP (**D**), hAPP/A β (**G**), and intracellular A β _{1–42} (**J**) were performed on each brain and are presented in histogram format. Error bars indicate SEM. * $P < 0.05$; *** $P < 0.001$.

and astrocytes, as well as microglia, can produce this proinflammatory cytokine in response to infection or other pathological stimuli (reviewed in Ref.²⁵). These more recent studies have shown that brain IFN γ expression is significantly induced in response to infections by herpes simplex virus-1,^{26,27} human herpesvirus 6,^{26,28} and *Chlamydia pneumoniae*.^{29–32} Moreover, increased pathogen burdens of each of these infectious agents have been associated, albeit controversially so, with heightened risk for development of sporadic AD.^{31,33} Such provocative findings raise the possibility that pathogen-induced IFN γ expression, which could feasibly occur at subclinical levels over a lifetime due to intermittent waves of latency and re-activation phases of infection, may serve as an active participant in disease-exacerbating and/or disease-ameliorating cellular processes.

The purported roles of IFN γ in the CNS are diverse, and our group is not the first to examine the effects of IFN γ overexpression in the context of the healthy or diseased mouse CNS. LaFerla et al³⁴ generated IFN γ transgenic mice and observed altered development of neuronally rich regions, such as the hippocampus and

cerebellum. These mice, which express IFN γ from a GFAP promoter-driven transcription unit, present with reduced developmental weights, death before sexual maturity, and a number of gross alterations in the hindbrain. Moreover, transgenic mice containing multiple copies of the IFN γ gene under the transcriptional control of the immunoglobulin A chain enhancer exhibit a number of skeletal developmental aberrations.³⁵ These prior studies, although they may provide insight into IFN γ activity/function within the CNS, make it evident that overexpression of IFN γ throughout embryogenesis results in aberrant development and potential induction of compensatory mechanisms that undoubtedly complicate interpretation of the action of this proinflammatory cytokine within the adult brain.

Perhaps more pertinent to our present study, Baron et al³⁶ used a similar transgenic approach for which they recently reported that mice arising from a cross between a transgenic line expressing IFN γ from an oligodendrocyte-specific promoter and an human APP-overexpressing mouse model of AD resulted in marked enhancement of neurogenesis within the dentate gyrus and improved

Figure 4. Tau pathology in 3 $\times$ Tg-AD mice is reduced by chronic IFN γ expression. Two-month-old 3 $\times$ Tg-AD mice ($N = 4$ to 5 per experimental group) were stereotactically infused bilaterally twice with 2 μ l ($\sim 3 \times 10^9$ transducing particles) of rAAV1-IFN γ or rAAV1-eGFP, and sacrificed 10 months later. The right hemisphere was processed for immunohistochemistry to detect human tau transgene product (HT7; **A** and **B**) or phospho-threonine 231 and phospho-serine 235 (AT180; **D** and **E**) or phospho-serine 202 (CP-13; **G** and **H**) of human tau. **Insets** in **B**, **E**, and **H** represent digitally magnified images of the respective outlined region in each photomicrograph for better visualization of stained cell morphology. Scale bar in **G** = 200 μ m. Quantitative image analyses for cells staining positive for HT7 (**C**), AT180 (**F**), and CP-13 (**I**) were performed on each brain and are presented in histogram format. Error bars indicate SEM. *** $P < 0.001$.

spatial learning and memory. Moreover, these compound transgenic mice showed evidence of modest inflammatory marker transcript increases, neuroprotection as measured by enhanced synaptophysin staining densities within the highly innervated CA1 and CA3 regions of the hippocampus, and suppression of oligodendrogenesis.

Effects of transgenic IFN γ expression on amyloid pathology severity in those mice were not reported. Despite the potential complications related to developmental compensation and effects on cellular composition within the brains of these compound transgenic mice during development, the report from Baron et al provides an informa-

A Changes in Steady-state Synaptic Marker Transcript Levels

Figure 5. Markers for enhanced neurogenesis increase in response to chronic IFN γ expression. Two-month-old 3 $\times$ Tg-AD mice ($N = 4$ to 5 per experimental group) were stereotactically infused bilaterally twice with 2 μ l ($\sim 3 \times 10^9$ transducing particles) of rAAV1-IFN γ or rAAV1-eGFP and sacrificed 10 months later. **A:** The left hemisphere was processed for total RNA isolation, and transcriptional marker levels for PSD-95 (Dlgh4), dynamin (Dnm1), and synaptophysin were analyzed via a PE7900HT quantitative real-time RT-PCR reaction. Relative fold changes in transcript levels are reported. Error bars indicate SEM. **B–G:** The right hemisphere was processed for immunohistochemistry to detect the mouse neuronal marker NeuN (**B** and **C**) or the neuroprogenitor marker DCX (**E** and **F**). **Insets** in **C** and **F** represent digitally magnified images of the respective outlined region in each photomicrograph for better visualization of stained cell morphology. Scale bar in **E** = 100 μ m. Quantitative image analyses for cells staining positive for NeuN (**D**) and DCX (**G**) were performed on each brain and are presented in histogram format. Error bars indicate SEM. ** $P < 0.01$; *** $P < 0.001$.

Figure 6. rAAV1-IFN γ -injected 3 $\times$ Tg-AD mice exhibit evidence of heightened neurogenesis within the transduced CA1 subregion of the hippocampus. Two-month-old 3 $\times$ Tg-AD mice ($N = 4$ to 5 per experimental group) were stereotactically infused bilaterally twice with 2 μ l ($\sim 3 \times 10^9$ transducing particles) of rAAV1-eGFP (**A**, **D**, **G**, **J**, and **L**) or rAAV1-IFN γ (**B**, **E**, **H**, **K**, and **M**). Mice received daily intraperitoneal injections of BrdU (50 mg/kg) for 3 days at 35 days after rAAV vector injection and were sacrificed 2 days after the final BrdU injection. **A** and **B**: diaminobenzidine immunohistochemical analysis for cells staining positive for BrdU incorporation was performed. **C**: The average numbers of BrdU-positive cells per microscopic field were quantified in the CA1 and dentate gyrus (DG) of the hippocampus and are presented in histogram format. Error bars indicate SEM. *** $P < 0.001$. Sections were co-incubated with a primary antibody specific for cell markers (red signal) for mature neurons (NeuN; **D** and **E**), neuronal progenitors (DCX; **G** and **H**), astrocytes (GFAP; **J** and **K**), or microglia (IBA-1; **L** and **M**), and a primary antibody for BrdU (blue signal). After incubation with a designated set of secondary antibodies, photomicrographs of the transduced CA1 subregion of the hippocampal formation were captured by two-color confocal microscopy. Overlapping signals appear pink. **Insets** in **B**, **E**, **H**, **K**, and **M** represent digitally magnified images of the respective outlined region in each photomicrograph for better visualization of stained cell morphology. Scale bars in **A** and **L** = 200 μ m. Quantitative image analyses for cells containing positive for NeuN and BrdU (**F**) and DCX and BrdU (**I**) were performed on each brain and presented in histogram format. Error bars indicate SEM. ** $P < 0.01$.

tive data set derived via independent means that strongly substantiates our findings that within the context of an AD-affected CNS, IFN γ conveys potent neuroprotective activity.

To circumvent the developmental impact of IFN γ on murine brain architecture and cell type composition, we used rAAV vectors to stereotactically and stably deliver IFN γ to an adult mouse brain genetically predisposed to developing AD-like amyloid and tau pathologies. As stated previously, brains of human AD patients and mouse models elicit a number of reactive changes, a subset of which includes inflammatory processes, as neurodegeneration progresses. The gene transfer vector chosen for studying IFN γ effects in the context of AD

pathogenesis should therefore ideally be devoid of inflammatory or immune stimulatory activity. rAAV vectors have been shown to be well tolerated in the human and rodent CNS, and, whenever they are introduced free of helper adenovirus, which was the case in the present study, measures of host inflammatory reaction are minimal or undetectable.^{37–40}

We had predicted that rAAV vector-mediated IFN γ expression in the 3 $\times$ Tg-AD mouse model would exacerbate both amyloid and tau pathologies, based on the marked signs of inflammation that were illustrated by increased transcript expression of cytokines, chemokines, and the microglia/macrophage marker CD11c, as well as the increase in numbers of IBA-1⁺ microglia

(Figure 2). Consistent with that hypothesis, intracellular A β 2 staining intensity was increased by 10 months of IFN γ expression within the hippocampi of 3 $\times$ Tg-AD mice (Figure 3). The increase in A β accumulation was not a consequence of altered expression of either the APP transgene or the endogenous mouse APP gene but possibly was due to modulated APP processing and/or A β secretion. In contrast, IFN γ overexpression exhibited a number of “neuroprotective” activities in this compound transgenic mouse model. For example, AT180 and CP-13 staining intensities for hyperphosphorylated tau were significantly depressed in IFN γ -overexpressing 3 $\times$ Tg-AD mice compared with those in rAAV-eGFP-infused control mice (Figure 4).

Hence, it seems that IFN γ represents a proinflammatory mediator with unique properties compared with those of other cytokines implicated in AD-related neuroinflammation. For example, whereas TNF- α promotes intracellular accumulation of A β 42 and eventual neuronal death when overexpressed in 3 $\times$ Tg-AD mouse brain,¹³ the proinflammatory cytokine IL-1 β seems to serve a neuroprotective role by enhancing the ability of microglia to prevent amyloid plaque pathology in a conditional mouse model of AD.⁴¹ Cytokines such as IL-1 and IL-6 have been shown to act through p38-mitogen-activated protein kinase and cdk5/p35-related signaling pathways, respectively, to enhance the pathogenic phosphorylation state of neuronal tau.^{42–44} Although untested, there is a possibility that IFN γ antagonizes these latter pathways or acts via a completely unrelated mechanism to suppress tau hyperphosphorylation. Signs of marked neurogenesis were also evident in IFN γ -expressing 3 $\times$ Tg-AD mice, a finding confirmed in a different murine AD model in the recent report from Baron et al.³⁶ IFN γ has been shown to promote neuronal differentiation *in vitro*,^{45,46} neuronal plasticity, and receptor clustering.⁴⁷ Moreover, human neural stem/progenitor cell cultures express the cognate receptors for IFN γ , termed IFN- γ R α and IFN- γ R β , and are driven toward a neuronal fate when cultured in the presence of IFN γ .⁴⁸ At present, we do not know whether the mechanisms underlying IFN γ -mediated tau hyperphosphorylation suppression and enhanced neurogenesis are related or are driven by disparate signaling cascades.

These observations, in aggregate, indicate that IFN γ evokes a number of potentially contradicting effects in the context of a brain undergoing AD-related degeneration, by apparently driving both disease-promoting and disease-ameliorating functions. Future experimentation will be centered on dissecting how these activities are mechanistically regulated and on whether functional outcomes are dependent on co-expressed cytokine/chemokine profiles, disease stage, and cellular milieu in which IFN γ is elaborated. A more complete understanding of the seemingly dichotomous activities of IFN γ may facilitate the development of therapeutic strategies designed to exploit the neuroprotective activity of this cytokine for degenerative conditions afflicting the brain.

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