

Intranasal interferon beta improves memory and modulates inflammatory responses in a mutant APP-overexpressing rat model of Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is the most common neurodegenerative disorder characterized by progressive cognitive decline. According to the critical role of inflammation in pathogenesis of AD and memory deficits, a cytokine with anti-inflammatory properties like interferon beta (IFN β), currently used to slow down disease progression and protect against cognitive disturbance in multiple sclerosis, might be also an effective treatment in AD condition. This study aimed to answer if the intranasal (IN) administration of IFN β with high CNS accessibility can alleviate memory impairments in a mutant APP-overexpressing rat model of AD through modulating inflammatory responses. To address this question, the lentiviruses carrying human amyloid protein precursor (APP) with the Swedish and Indiana mutations (LV-APP_{Sw/Ind}) were bilaterally injected in the hippocampus of adult rats. Memory performance was assessed using passive avoidance task on days 49 and 50 after injection. Moreover, the expression of glial markers (GFAP and Iba1) and pro-inflammatory (TNF- α , IL-1 β and IL-6) and anti-inflammatory cytokines (IL-10) were evaluated in the hippocampus. Therapeutic effects of IN-administered IFN β (0.5 μ g/kg and 1 μ g/kg doses, every other day from day 23 to 50 after lentivirus injection) were examined in the LV-APP-injected rats. Our results showed that over-expression of mutant human APP gene in the hippocampus led to learning and memory deficits concomitant with gliosis and pro-inflammatory responses. Interestingly, treatment of AD-modeled rats with IFN β ameliorated memory impairments possibly through suppressing gliosis and shifting from pro-inflammatory toward anti-inflammatory status, suggesting that IFN β may be a promising therapeutic agent to improve cognitive functions and modulate inflammatory responses in an AD-like neurodegenerative context.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease which is clinically characterized by memory decline and cognitive dysfunction and affects more than 44 million individuals in the

world. Currently, sporadic late-onset AD including more than 95% of patients usually develops in persons aged 65 years and over, with incidence doubling every 5 years. In contrast, early-onset AD comprises around 5% of cases and appears before age 65 (Bekris et al., 2010; Masellis et al., 2013). Familial early-onset forms of AD accounting for

Abbreviation: AD, Alzheimer's disease; APP, amyloid-precursor-protein; AICD, APP intracellular domain; AAV, adeno-associated virus; A β , β -amyloid; β -CTF, C-terminal fragment; BBB, blood-brain barrier; (CNS), central nervous system; DAB, 3,3'-Diaminobenzidine tetrahydrochloride; EAE, experimental autoimmune encephalomyelitis; eGFP, enhanced green fluorescent protein; GFAP, glial fibrillary acidic protein; hAPP, human amyloid precursor protein; Iba1, ionized calcium-binding adapter molecule1; IF, immunofluorescence; IHC, immunohistochemistry; IN, intranasal; IFN β , interferon beta; IFNAR, type I IFN receptor; IFN-I, type I interferon; IFN α , interferon alpha; IL-1 β , interleukin-1 β ; IL-6, interleukin-6; IL-10, interleukin-10; JAK1, Janus kinase 1; LV-hAPP_{Sw/Ind}, lentivirus encoding human APP with Swedish and Indiana mutations; LV, lentivirus encoding eGFP; LTM, long-term memory; NGS, normal goat serum; OCT, optimal cutting temperature; PS, presenilin; PBS, phosphate buffer saline; PA, Passive avoidance test; PFA, paraformaldehyde; PBST, PBS containing Tween 20; PD, Parkinson's disease; qPCR, quantitative polymerase chain reaction; RRMS, relapsing-remitting multiple sclerosis; STM, short-term memory; sAPP β , soluble APP; STAT1, signal transducer and activator of transcription 1; STAT3, signal transducer and activator of transcription 3; SCI, spinal cord injury; TNF- α , tumor necrosis factor- α ; TYK2, tyrosine kinase 2

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around 1% of all AD cases are mostly induced by mutations in the presenilin (PS) or amyloid-precursor-protein (APP) genes (Lanoiselée et al., 2017; Shea et al., 2016). It is widely accepted that extracellular β -amyloid ($A\beta$) plaques and intracellular neurofibrillary tangles, formed by hyperphosphorylated tau proteins are the main neuropathological hallmarks in both early- and late-onset forms of AD (Serrano-Pozo et al., 2011). $A\beta$ plaques are primarily composed of $A\beta$ peptide which is generated through proteolytic cleavage of APP (Chow et al., 2010; O'Brien and Wong, 2011). APP is firstly cleaved by β -secretase to yield soluble APP (sAPP β) and C-terminal fragment (β -CTF). Then, γ -secretase cleaves the β -CTF into APP intracellular domain (AICD) and $A\beta$ peptide (Tamaye et al., 2012). Although $A\beta$ accumulation in the brain plays a critical role in pathogenesis of AD, several studies have demonstrated that memory dysfunction and neuropathological events are initiated before $A\beta$ plaque formation (Hamm et al., 2017; Heneka et al., 2005; Lodeiro et al., 2017; Wright et al., 2013). Recently, it has been indicated that APP metabolites are involved in cognitive impairments and AD pathology independently of $A\beta$ (Hamm et al., 2017). Further, the elevated levels of APP fragments have been previously shown in the brain of AD patients (Kametani and Hasegawa, 2018). It has been revealed that APP fragments trigger production of pro-inflammatory cytokines in APP transgenic mice.

Neuroinflammation is one of the earliest neuropathological events in AD which contributes to early cognitive deficits in APP_{Sw/Ind} transgenic mice (Wright et al., 2013) and AD patients (Monson et al., 2014; Parachikova et al., 2007). It has been reported that pro-inflammatory mediators cause an increase in the activity of β -secretase and then APP processing into $A\beta$ (Sastre et al., 2008). $A\beta$ interacts with various microglial receptors, including Toll-like receptors, receptors for advanced glycosylation end products and scavenger receptors (Doens and Fernández, 2014). As a result, $A\beta$ stimulates expression of pro-inflammatory cytokines (Doens and Fernández, 2014; Wei et al., 2016) and ultimately leads to cognitive decline in AD (Hanna et al., 2012). Thus, strategies to modulate neuroinflammation in the early stage of AD can prevent its progression and ameliorate the symptoms of the disease (Imbimbo et al., 2010).

IFN β , a member of the type I interferon (IFN-I) family, is the most common treatment for relapsing-remitting multiple sclerosis (RRMS) patients (Comabella et al., 2009). Although peripheral effects of IFN β have been studied more, it possesses essential effects in the central nervous system (CNS). In the CNS, both neurons and glial cells are known to produce and respond to IFN β (Liu et al., 2013; Pfefferkorn et al., 2015; Reinert et al., 2016; Zhang et al., 2014). Numerous studies have reported the expression of IFN β receptors in the hippocampus, cerebral cortex, striatum, olfactory bulbs, and cerebellum (Costello and Lynch, 2013; Ling et al., 2014; Ross et al., 2004). IFN β binds to the type I IFN receptor (IFNAR), consisted of two subunits including IFNAR1 and IFNAR2. Dimerization of the IFNAR leads to the activation of Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2), followed by the phosphorylation and dimerization of signal transducer and activator of transcription 1 (STAT1) and STAT3. STAT1 and STAT3 homodimers translocate to the nucleus and consequently transcribe target genes (Ivashkiv and Donlin, 2014). The baseline level of IFN β is shown to have a critical effect on the survival of hippocampal neurons, neurite outgrowth, dendritic branching, neurogenesis and cognitive learning (Ejlertskov et al., 2015). Recently, the protective effects of exogenous IFN β have been widely reported in the animal models of Parkinson's disease (PD) (Ejlertskov et al., 2015), spinocerebellar ataxia (Chort et al., 2013), stroke (Dixon et al., 2016; Kuo et al., 2016; Liu et al., 2002; Veldhuis et al., 2003), spinal cord injury (SCI) (Beta, 2013; Gok et al., 2007; Nishimura et al., 2013; Sandrow-Feinberg et al., 2010) as well as experimental autoimmune encephalomyelitis (EAE) (Aritake et al., 2010; Hamana et al., 2017; Kocur et al., 2015; Liu et al., 2010; Marin-Bañasco et al., 2017; Mohammadzadeh et al., 2016). Some of the mechanisms underlying IFN β effects include a shift from pro- to anti-inflammatory cytokines production (Lubina-Dąbrowska et al., 2017),

secretion of growth factors (Biernacki et al., 2005), inhibition of gliosis (Nishimura et al., 2013) and neuronal apoptosis (Dixon et al., 2016), degradation of mutant proteins via promoting autophagy (Chort et al., 2013; Ejlertskov et al., 2015), preservation of BBB integrity and thereby limiting immune cells infiltration into the CNS (Hojati et al., 2016).

In the context of AD, there is only one study investigating the safety and efficacy of subcutaneous injection of IFN β 1a in early AD patients. The treatment was shown to be safe and tolerable, causing significant improvement in the daily activity of patients. However, a partial but not significant amelioration of cognitive deficits was reported in these IFN β 1a-treated AD patients (Grimaldi et al., 2014). It is noticeable that in systemic administration routes (i.e., intravenous, subcutaneous, etc.), a small fraction of exogenous IFN β penetrates into the CNS due to its short half-life in serum (Basu et al., 2006), development of neutralizing antibodies in the periphery (Pachner et al., 2009) and its limited ability to cross the BBB (Ross et al., 2004). For these reasons, the intranasal (IN) administration of protein drugs which offers a non-invasive and efficient route along the olfactory and trigeminal neural pathways that bypasses the BBB, and delivers large molecules directly to different brain structures (Crowe et al., 2018; Hanson et al., 2013) is of particular interest. The IN approach has been previously tested for IFN β and shown its delivery to cortex and hippocampus (Ross et al., 2004; Thorne et al., 2008). The present study was designed to investigate the effects of IN administration of IFN β in a mutant human APP-induced rat model of AD. Memory performance, glial activation as well as expression of pro- and anti-inflammatory cytokines were the outcomes evaluated.

2. Materials and methods

2.1. Cloning and lentiviral particles preparation

AD animal model was generated using delivery of lentivirus encoding human APP with Swedish (K670N/M671L) and Indiana (V717F) mutations (LV-hAPP_{Sw/Ind}) in the hippocampus of adult rats according to our previous work (Parsi et al., 2015). Briefly, mutant human APP cDNA that cloned in pcDNA3.1/Zeo was kindly provided by Dr. Lars N.G. Nilsson. To amplify APP sequence, a pair of primers (forward primer 5'-TTACCGGTGCGATGCTGCCCGTTTGGC-3', and reverse primer 5'-CCCCTAGCCTAGTCTGCATCTGCTCAAAG-3') was designed and synthesized based on the human APP sequence (NM_000484.3). The forward primer contains an AgeI site, while the reverse primer includes a NheI site for subsequent subcloning. PCR conditions were carried out according to Taq Plus PCR MasterMix (Yekta Tajhiz Azma, Iran, #YT1553) manufacturer's protocol as follows: pre-denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 2 min; and a final extension at 72 °C for 7 min. After purification, amplified APP fragment was digested with AgeI (Thermo Scientific, USA, #ER1461) and NheI (Thermo Scientific, USA, #ER0975) restriction enzymes and ligated into the corresponding restriction sites of FSynIGW lentiviral vector (gifted from G. Morfini, University of Illinois) to develop FSynIGW-APP_{Sw/Ind} expression vector. APP sequence in downstream of synapsin, as a neuronal specific promoter, was tagged with an internal ribosome entry site (IRES)-enhanced green fluorescent protein (eGFP) sequence. The expression vector was verified by digestion and sequencing. Viral particles were constructed by lentiX-293 T cells (Clontech, USA, #632180) using virapower packaging mix (Invitrogen, USA, #k497000). The manufacturer's instruction was followed to produce viral particles. 48 h after transfection, culture supernatant containing virus particles was harvested, filtered with a 0.45 μ m PVDF filter (Jet biofil, China) and finally ultracentrifuged at 50 \times 10³g for 2.5 h. Then, the lentiviral pellet was dissolved in the phosphate buffer saline (PBS), aliquoted and stored at -80 °C until use. Viral titer was assessed by flow cytometry (Drayman and Oppenheim, 2011). Titer was approximately (10⁹) TU/mL. The lentivirus expressing only eGFP was used as a control (LV).

2.2. Animals

Adult male Wistar rats (240–260 g body weight) were obtained from our breeding colony (Neuroscience Research Center) and housed in groups of four per cage. All animals were maintained under standard laboratory conditions (a 12 h light/dark cycle, the temperature of $21 \pm 2^\circ\text{C}$) with free access to food and water. All experiments were carried out based on the guide for the care and use of laboratory animals (National Institutes of Health Publication No. 80-23, revised 1996) and confirmed by the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.PHNS.REC.1395.61).

2.3. Intracerebral injection of the lentiviral suspension

Rats were intraperitoneally anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (20 mg/kg) and placed into a stereotaxic apparatus (Stoelting, USA). After the surgery, 2.5 μL of the concentrated LV-APP suspension was bilaterally injected into the CA1 area of the hippocampus (AP: -3.84 mm, ML: ± 2.2 mm, DV: -2.5 mm based on the Paxinos and Watson atlas (Paxinos and Watson, 2007) using 30-gauge needle connected to a Hamilton syringe through a polyethylene tubing at a rate of 0.5 $\mu\text{L}/\text{min}$ (Parsi et al., 2015). Animals in the control group received the same volume of the LV. In order to assess the transduction efficiency, the control and AD-modeled animals ($n = 3/\text{group}$) were sacrificed on day 50 after injection. For this purpose, expression of GFP and APP transgenes in the hippocampus was confirmed by immunofluorescence (IF) staining and quantitative polymerase chain reaction (qPCR) analysis and immunohistochemistry (IHC) staining, respectively. Furthermore, A β deposits known as a landmark for AD was evaluated in the hippocampus using thioflavin T and IHC staining.

2.4. Experimental design and drug administration

After verification of the transduction efficiency and AD modeling, treatment with IN IFN β was started. Rats were randomly assigned into six experimental groups ($n = 10/\text{group}$):

(1) Control group (LV): Rats received the LV intrahippocampal injection and IFN β vehicle (deionized water) intranasal administration.

(2) AD group (LV-APP): Rats received the LV-APP intrahippocampal injection and IFN β vehicle intranasal administration.

(3, 4) Treatment groups (LV-APP + IFN β (0.5 $\mu\text{g}/\text{kg}$), LV-APP + IFN β (1 $\mu\text{g}/\text{kg}$)): Rats received the intrahippocampal injection of the LV-APP and also were intranasally treated with two different doses of IFN β (0.5 $\mu\text{g}/\text{kg}$ and 1 $\mu\text{g}/\text{kg}$).

(5, 6) IFN β groups: Rats received the LV intrahippocampal injection and intranasal administration of two different doses of IFN β (0.5 $\mu\text{g}/\text{kg}$ and 1 $\mu\text{g}/\text{kg}$).

Human recombinant IFN β 1a (ReciGen[®]), from CinnaGen Co. (Tehran, Iran), was diluted with cold deionized water to a volume of 5 $\mu\text{L}/\text{rat}$ to prepare the doses of 0.5 $\mu\text{g}/\text{kg}$ ($\sim 34,000$ IU/rat) and 1 $\mu\text{g}/\text{kg}$ ($\sim 68,000$ IU/rat). The IN IFN β doses were chosen based on the previous study that revealed the protective effects of IN administration of human IFN β 1a in a rat model of hypoxic-ischemic injury (Dixon et al., 2016). According to our experimental protocol as shown in Fig. 1, rats were intranasally treated with IFN β doses every other day from day 23 to 50 after lentivirus injection (14 doses). IN IFN β delivery was carried out under mild anesthesia while the rat was laid on his back and the head was maintained in a horizontal position during the delivery. A 5 μL volume of IFN β was delivered into the nostrils (2.5 $\mu\text{L}/\text{nostril}$) by insertion of a polyethylene 10 tube attached to a Hamilton syringe (Bender et al., 2015). The same volume of IFN β vehicle was administered to the LV and LV-APP animals. In order to evaluate memory performance, rats in all experimental groups were subjected to passive avoidance test on days 49 and 50 after injection (Parsi et al., 2015). After completion of the behavioral test, animals of the control, AD-modeled and treatment groups were sacrificed. The hippocampus tissue

was immediately separated, frozen in liquid nitrogen and kept at -80°C until quantitative polymerase chain reaction (qPCR) analysis ($n = 3\text{--}4/\text{group}$).

2.5. Passive avoidance test (PA)

The passive avoidance task is a fear-motivated tests used to evaluate hippocampus-dependent non-spatial memory in different AD models (Kim et al., 2014; Senechal et al., 2008). We performed the passive avoidance test at day 49 after lentivirus injection in accordance with the protocol described previously (Beirami et al., 2018). Passive avoidance apparatus (shuttle box) composed of light and dark compartments of the same size (30 \times 20 \times 20 cm) separated by a guillotine door (10 \times 20 cm). Briefly, on the training trial followed 30 min after habituation trial, rat was put in the light chamber and then the door was opened. After the rat entered the dark chamber, the door was closed and immediately delivered a foot-shock (50 Hz, 1 mA, and 2 s) via electrified steel rods in the floor of the box. Next, the retention trials were assessed 90 min and 24 h after training trial and considered as short-term memory (STM) and long-term memory (LTM), respectively. The retention trials were done in the same way as training trial with exception that no foot shock was applied and the latency into the dark compartment (step-through latency) was recorded in a period of 300 s as an index of memory retrieval.

2.6. RNA extraction and qPCR

Total RNA was isolated from the hippocampus based on YTzol (Yekta Tajhiz Azma, Iran, #YT9063) manufacturer's instruction, and subsequently eluted in 30 μL of RNase-free water. Purity and concentration of all the extracted RNA samples were measured by NanoDrop 2000 (Thermo Scientific, USA) prior to cDNA synthesis. Moreover, extracted RNAs were converted to cDNA using PrimeScript[™] RT reagent kit (Takara, Japan, #RR037A). 10 μL reactions were set up following manufacturer's recommendations using 500 ng of total RNA and then incubated at 37°C for 15 min and at 85°C for 5 s. Next, sample cDNA was used as a template for qPCR reaction to quantify the expression of human amyloid precursor protein (hAPP), glial fibrillary acidic protein (GFAP), ionized calcium-binding adapter molecule1 (Iba1), tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6) and interleukin-10 (IL-10) genes. Specific primers were designed using Allele ID 7, as presented in Table 1. qPCR was performed by SYBR Green qPCR Master Mix (2 \times) (Ampliqon, Denmark) and ABI StepOne (Applied Biosystems, USA) instrument under cycling conditions: activation at 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. All genes were normalized to β -actin level. The $2^{-\Delta\Delta\text{Ct}}$ method was used to quantify mRNA levels of the genes mentioned above (Livak and Schmittgen, 2001). In addition, hAPP and β -actin reaction products were electrophoresed on ethidium bromide-stained agarose gel and then the corresponding bands were visualized.

2.7. Histochemistry

In this study, following transcardial perfusion of anesthetized rats with ice-cold PBS (0.1 M; pH 7.4) and 4% paraformaldehyde (PFA), the brains were harvested, post-fixed in PFA overnight, and cryoprotected in 30% sucrose for 48 h at 4°C . The brains were then embedded, frozen in OCT (optimal cutting temperature) compound (Bio optica, Italy, #059801), and then kept at -80°C until sectioning. Frozen coronal sections (10 or 20 μm thickness) were prepared using a cryostat apparatus (Scilab, UK) along the anterior-posterior axis of the hippocampus (AP: -3.2 to -4.2 mm relative to bregma) and placed on gelatin-coated slides. The sections were then used for histology assays.

For IHC staining, the sections were washed and rehydrated three times in PBS for 5 min each time, and then endogenous peroxidase activity was quenched with 3% hydrogen peroxide (Merck, Germany)

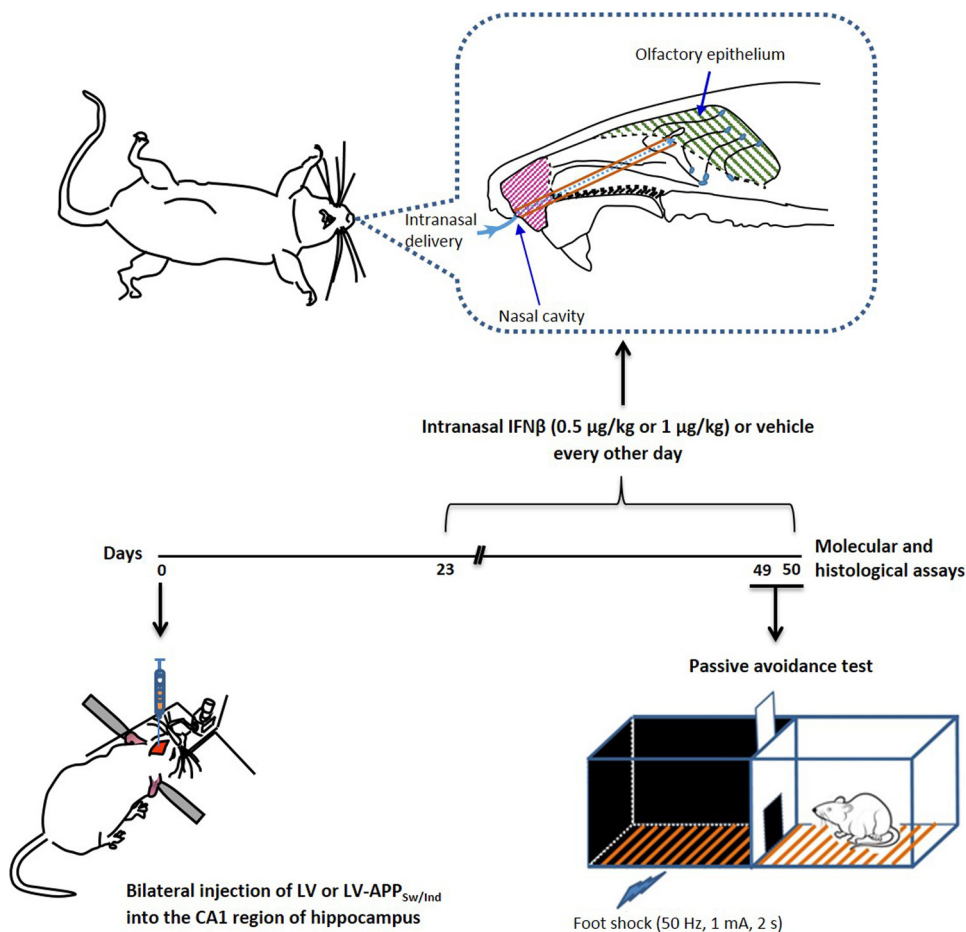


Fig. 1. The timeline of experiments. Lentiviruses encoding human amyloid precursor protein (APP) with Swedish and Indiana mutations (LV-APP_{Sw/Ind}) and the control construct (LV) were bilaterally injected in the hippocampus of adult rats. From day 23 after injection, rats were intranasally treated with interferon beta (IFN β) doses (0.5 μ g/kg or 1 μ g/kg) every other day until day 50. On days 49 and 50, rats were subjected to passive avoidance test. Animals were sacrificed on day 50 and the hippocampus tissue was used for molecular and histological assays.

for 10 min. After permeabilization by 1% Triton X-100 (Merck, Germany) for 10 min, non-specific bindings were blocked with 1 h incubation in blocking buffer (10% normal goat serum (NGS, Sigma, USA) in PBST (PBS containing 0.05% Tween 20 (Merck, Germany))). Finally, the sections were treated overnight with primary antibody (diluted in PBST with 5% NGS) at 4 °C. The following primary antibodies were used: rabbit anti-amyloid precursor protein (1:50, Abcam, USA, #ab15272) and rabbit anti-beta amyloid 1–42 (1:50, Abcam, USA, #ab10148). On the second day, the sections were incubated with the HRP-linked secondary antibody (EnVisionTM+ Dual Link System-HRP, Dako Denmark A/S, Glostrup, Denmark) for 1 h at room temperature. Immunoreactivities were then visualized by treatment with 3, 3'-Diaminobenzidine tetrahydrochloride (DAB) solution followed by nuclear counterstaining with hematoxylin. After dehydration using an ascending series of ethanol, the sections were cleared in xylene, and then cover-slipped. Images were obtained by the light microscope (Nikon).

For IF staining, the sections were washed and rehydrated three

times in PBS for 5 min each time. Subsequently, antigen retrieval was performed by immersing the slides in citrate buffer (pH = 6) for 30 min at 80 °C in a water bath. After have been cooled for an additional 30 min, the sections were permeabilized by 1% Triton X-100 for 10 min, and incubated in the blocking buffer for 1 h at room temperature. Following this, the sections were incubated overnight at 4 °C with primary antibody against GFP (1:100, Abcam, USA, #ab290) diluted in PBST containing 5% NGS. On the next day, immunoreactivity was detected and visualized after being incubated the sections in goat anti-rabbit IgG FITC conjugated (1:50, Sigma, USA, #F1262) for 1 h at room temperature and followed by nuclear counterstaining with DAPI reagent (Sigma, USA). Images were then obtained by the fluorescence microscope (Olympus).

Thioflavin T staining for the detection of A β aggregates was performed as previously described (Picken and Herrera, 2012). Here, the sections were washed and rehydrated three times in PBS for 5 min each time, stained with fresh, 1% filtered thioflavin T (Sigma, USA, #T3516) for 10 min, and finally washed sequentially in PBS and deionized water.

Table 1
Primer sequences used in qPCR.

Gene	Forward primer (5'–3')	Reverse primer (5'–3')
Human APP	TGCCCACTGGCTGAAGAAAGTGAC	ACCTCATCACCATCCTCATCGTCC
GFAP	AACCGCATCACCAITCCTGT	TCCTTAATGACCTCGCCATCC
Iba1	TCGTCACTCCCCACCTAAG	ATCAAACCTCCATGTACTCGTCTTG
TNF- α	ACTGAACTTCGGGGTGATCG	CGCTTGGTGGTTTGCTACG
IL-1 β	ACCCAAGCACCTTCTTTCCCTC	GTCGTTGCTTGTCTCTCCTTGTA
IL-6	GTATGAACAGCGATGATGCACTG	CATTGGAAGTTGGGGTAGGAAGG
IL-10	GAAGCTGAAGACCCTCTGGATAC	CTCATTATGGCCTTGTAGACACC
β -actin	TCTATCCTGGCTCACTGTC	AACGCAGCTCAGTAACAGTCC

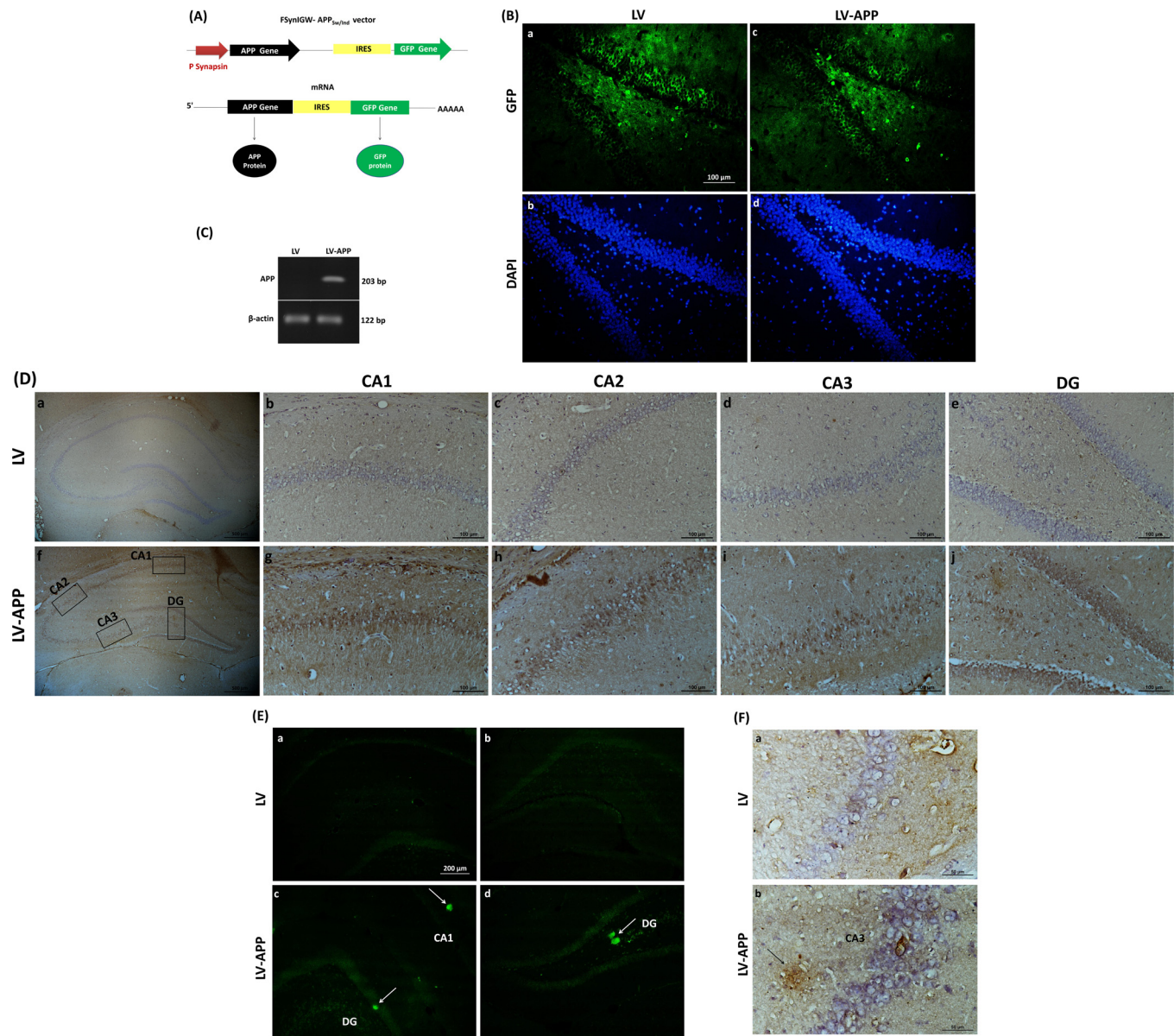


Fig. 2. Lentiviral-mediated expression of green fluorescent protein (GFP), amyloid precursor protein (APP) and amyloid beta (A β) in the hippocampus. (A) Schematic image of bicistronic FSynIGW-APP_{Sw/Ind} lentiviral vector. (B) GFP immunoreactivity was detected predominantly in the dentate gyrus cells of the hippocampus in both LV (a, b) and LV-APP-injected rats (c, d). Scale bar: 100 μ m. (C) The expression of human APP gene was only found in the LV-APP group. (D) An elevated expression of APP protein in all hippocampal subfields including CA1, CA2, CA3 and DG was observed in the LV-APP group (f–j) compared with the LV group (a–e). Scale bars: 500 μ m and 100 μ m. (E, F) Thioflavin T and immunohistochemistry stainings indicate that A β plaques were only identified in the hippocampus of the LV-APP-received rats (E (c, d), and F (b)) but not in the LV-received rats (E (a, b), and F (a)). Scale bars: 200 μ m and 50 μ m. n = 3/group.

Images were then obtained by the fluorescence microscope (Olympus). 20 μ m-thick sections were used for A β IHC and thioflavin T stainings.

2.8. Statistical analysis

All Data were analyzed using 16th version of SPSS software. One-way ANOVA followed by post hoc Tukey's test was applied to compare differences between the groups. $P < 0.05$ was considered statistically significant. Data were reported as the mean \pm SEM.

3. Results

3.1. Lentivirus-mediated expression of GFP, APP, and A β was detected in the hippocampus

We first investigated whether GFP and human APP could be efficiently expressed in the hippocampus following transduction. Using IF staining, GFP expression was examined in the LV and LV-APP groups on day 50 after lentivirus injection. Interestingly, in both experimental groups, GFP was predominantly expressed in the dentate gyrus cells (Fig. 2B). In addition, scattered green cells were observed in the other regions of the hippocampus (data not shown). Similarly, Ahmed et al have reported that the dentate gyrus neurons are more frequently transduced by lentivirus than other neurons in the rodent hippocampus (Ahmed et al., 2004). Another study has also shown that GFP transgene

is strongly expressed in the dentate gyrus cells after injection of lentivirus into the hippocampal CA1 area of the mouse (Osinde et al., 2008). We further investigated mutant human APP expression by qPCR and IHC. qPCR analysis using human APP specific primers confirmed the expression of hAPP gene only in the LV-APP experimental group (Fig. 2C). In IHC staining, we observed an elevated expression of APP protein in the hippocampus of the LV-APP-received rats compared to the LV-received rats (Fig. 2D). Interestingly, APP immunoreactivity was not only present around the injection site (CA1) but also diffusely distributed and existed throughout the hippocampal subfields including dentate gyrus, CA2, and CA3 (Fig. 2D). Despite the fact that GFP expression level was lower than APP expression level, the lentiviral system used in this study resulted in an effective and widespread transduction of hippocampal neurons. Difference observed in expression of GFP and APP genes may be due to the particular position of these genes in Fsyn1GW lentiviral vector. As shown in Fig. 2A, APP gene sequence is upstream of IRES2-GFP sequence. IRES2 sequence allows bicistronic expression of APP and GFP under a neuron-specific promoter, synapsin. It has been indicated that transduced cells with a variety of bicistronic LV strongly express upstream gene of IRES sequence, whereas GFP expression (downstream gene of IRES sequence) is very low or undetectable in these cells (Yu et al., 2003).

Accumulation of A β deposits is a pathological hallmark in AD patients and animal models. 50 days after the LV-APP injection, we observed low levels of A β plaques only in the hippocampus of the LV-APP-received rats following IHC and thioflavin T stainings (Fig. 2E, F, respectively). In line with our results, it has been reported that 2-month-old APP_{Sw/Ind} transgenic mice exhibit low levels of A β in the hippocampus (Hamm et al., 2017).

3.2. IFN β intranasal administration ameliorated memory impairments induced by the LV-APP

After AD modeling, we assessed the effects of IFN β IN administration on memory deficits induced by intrahippocampal injection of LV-APP. As depicted in the Fig. 3A and B, a significant reduction of the step-through latency in the STM ($F_{5, 54} = 11.396$, $P < 0.001$) and the LTM ($F_{5, 54} = 6.306$, $P < 0.001$) sessions was found in the LV-APP group compared to the LV group, indicating short-term and long-term memory impairments. Interestingly, IN administration of IFN β improved the memory performance. As shown in the Fig. 3A, LV-APP-injected rats that were intranasally treated with 0.5 μ g/kg or 1 μ g/kg

dose of IFN β exhibited a significant increase in the step-through latency in the STM session compared with the LV-APP-injected rats (Fig. 3A, $P < 0.001$). Also, in the LTM session, IFN β -treated rats showed a significantly longer step-through latency than the LV-APP-received rats (Fig. 3B, $P < 0.01$ for the lower dose of IFN β and $P < 0.001$ for its higher dose). Specifically, in both sessions, rats that received only IFN β did not show a significant difference in the step-through latency comparison with the LV group.

3.3. IFN β intranasal administration modulated inflammatory responses induced by the LV-APP

It has been demonstrated that neuroinflammation is associated with the memory impairments (Morales et al., 2014). In the CNS, glial cells produce and release a wide range of pro and anti-inflammatory mediators (Rai et al., 2014). Our results indicated that the mRNA expression levels of GFAP (an astrocyte marker) and Iba-1 (a microglia marker) genes significantly increased in the LV-APP group compared with the LV group (Fig. 4A and B, $P < 0.05$, $P < 0.01$, respectively). Interestingly, the treatment with both doses of IFN β significantly reduced the mRNA expression of GFAP (Fig. 4A, $P < 0.01$) and Iba1 (Fig. 4B, $P < 0.05$ for the lower dose of IFN β and $P < 0.01$ for its higher dose) genes in the hippocampus of the LV-APP-injected rats.

The one-way analysis revealed that the mRNA expression levels of TNF- α , IL-1 β , and IL-6 genes significantly increased in the LV-APP group compared to the LV group (Fig. 4C, D, and E, $P < 0.01$, $P < 0.05$, $P < 0.001$, respectively). In contrast, the LV-APP-injected rats that received either lower or higher-dose of IFN β showed a significant decrease in TNF- α mRNA expression level compared to the LV-APP group (Fig. 4C, $P < 0.05$). Furthermore, our results indicated that only the LV-APP-injected rats treated with higher dose of IFN β exhibited a significant decrease in IL-1 β and IL-6 mRNA levels compared with the LV-APP group (Fig. 4D and E, $P < 0.05$, $P < 0.01$, respectively).

In addition, the mRNA expression level of interleukin-10 (IL-10) gene, as an anti-inflammatory cytokine (Rai et al., 2014), was analyzed in the hippocampus of all experimental groups. As evidenced in the Fig. 4F, the expression level of IL-10 decreased in the LV-APP group compared to the LV group, however the change was not statistically significant ($P > 0.05$). The mRNA level of IL-10 significantly increased following the treatment with both doses of IFN β compared with the LV-APP group (Fig. 4F, $P < 0.05$).

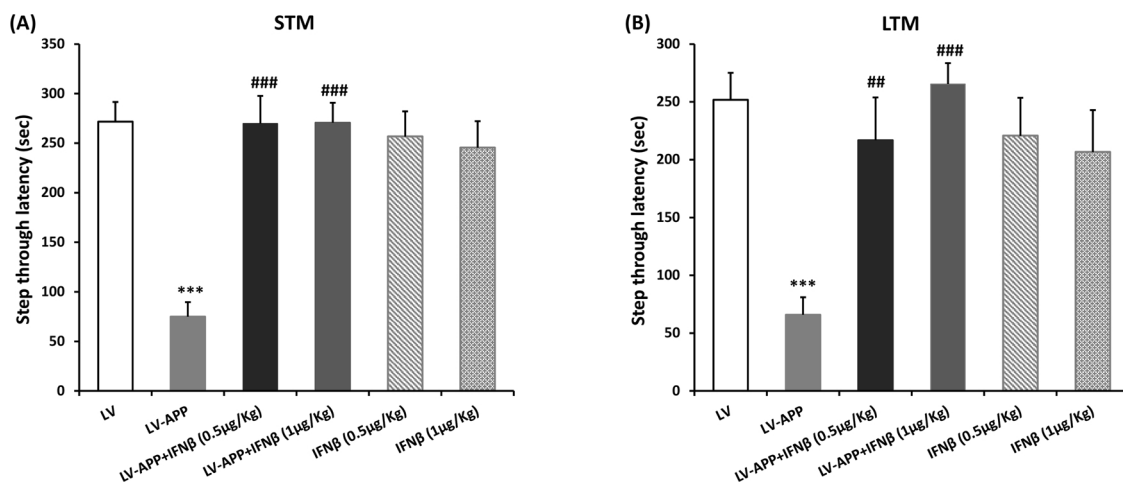


Fig. 3. The effect of intranasal administration of interferon beta (IFN β) on memory impairments induced by intrahippocampal injection of the LV-APP. (A, B) Treatment with both doses of IFN β (0.5 μ g/kg or 1 μ g/kg) ameliorated short and long-term memory deficits induced by the LV-APP injection in the passive avoidance task. (A) The first retention trial was examined 90 min after the acquisition trial (short-term memory; STM). (B) The second retention trial was assessed 24 h after the acquisition trial (long-term memory; LTM). Data are reported as the mean \pm SEM. $n = 10$ /group; the differences between groups were determined by ANOVA followed by Tukey test. *** $P < 0.001$ versus the LV group, ## $P < 0.01$ and ### $P < 0.001$ versus the LV-APP group.

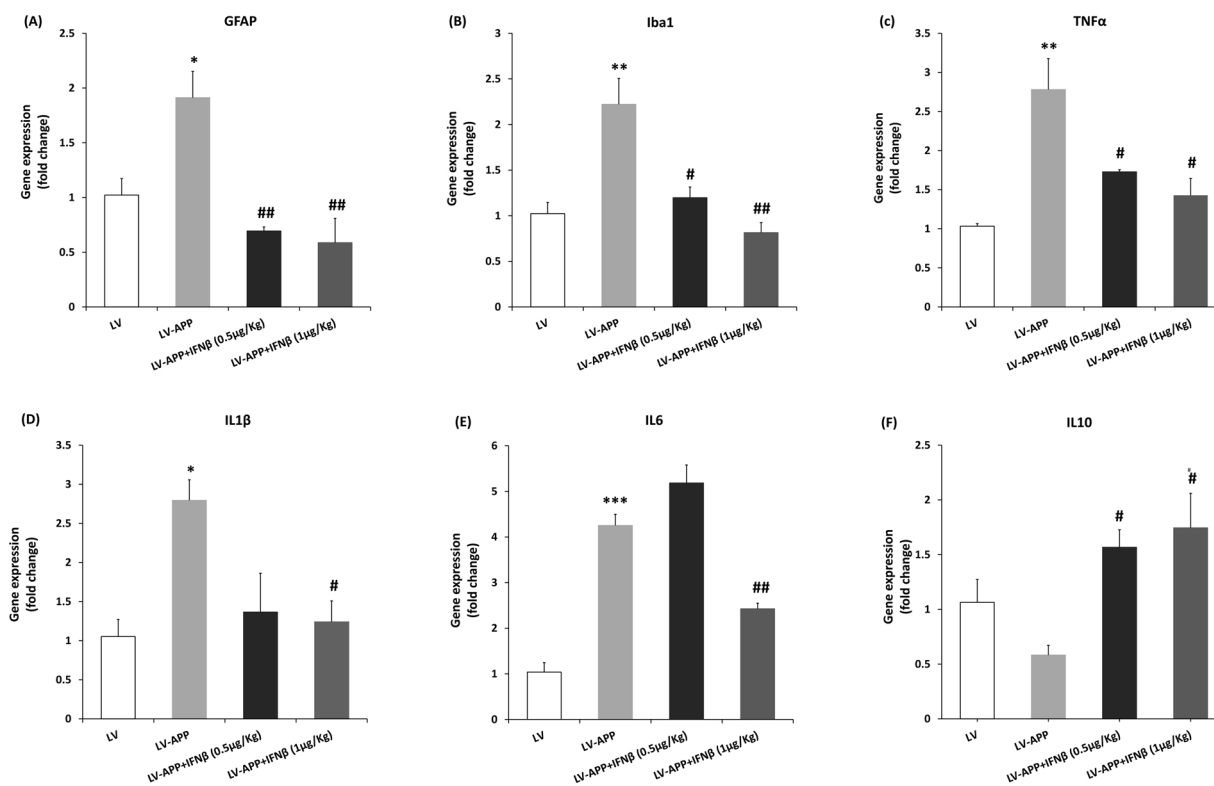


Fig. 4. The effect of intranasal administration of interferon beta (IFN β) on glial activation and inflammatory responses induced by intrahippocampal injection of the LV-APP. IFN β intranasal administration resulted in suppression of gliosis and shift from pro- to anti-inflammatory cytokines production. The higher dose of IFN β (1 μ g/kg) can inhibit pro-inflammatory responses more effectively than its lower dose (0.5 μ g/kg). Quantitative polymerase chain reaction analysis of the expression of (A) glial fibrillary acidic protein (GFAP), (B) ionized calcium-binding adapter molecule1 (Iba1), (C) tumor necrosis factor- α (TNF- α), (D) interleukin-1 β (IL-1 β), (E) interleukin-6 (IL-6) and (F) interleukin-10 (IL-10) genes in the hippocampus of all experimental groups. Data are reported as the mean \pm SEM. n = 3–4/group; the differences between groups were determined by ANOVA followed by Tukey test. *P < 0.05, **P < 0.01 and ***P < 0.001 versus LV group; #P < 0.05 and ##P < 0.01 versus LV-APP group.

4. Discussion

Our results showed that the over-expression of mutant human APP_{Sw/Ind} in the hippocampus induced short-term and long-term memory impairments concomitant with the increased levels of mutant APP protein and A β plaques in the CA1, CA3 and DG regions of the AD-modeled rats. These results are consistent with our previous work (Parsi et al., 2015) concerning memory deficits induced by over-expression of the mutant human APP_{Sw/Ind} in the rat hippocampus. Although we observed memory impairments at behavioral level, but low levels of A β plaques were detected in the hippocampus. In line with this finding, Hamm et al have recently shown that 2-month-old APP_{Sw/Ind} transgenic mice exhibit early memory deficits despite the low levels of A β , which is related to accumulation of APP fragments in the hippocampus (Hamm et al., 2017). Several studies have reported that transgenic mice with increased levels of sAPP β , β -CTF (Tamayev et al., 2012) and AICD (Ghosal et al., 2009) in the brain develop cognitive dysfunction and AD-like pathological features. In addition, high levels of APP fragments have been found in the brain of AD patients which contribute to pathogenesis of sporadic and familial forms of AD (Kametani and Hasegawa, 2018). So far, there is no effective treatment to slow down or cure cognitive decline in AD. It has been reported that neuroinflammation plays critical roles in A β plaque formation, memory deficits and progression of AD (Monson et al., 2014; Morales et al., 2014; Parachikova et al., 2007; Sastre et al., 2008; Wright et al., 2013; Zhang et al., 2012). Clinical studies have revealed neuroprotective effects of anti-inflammatory drugs in early stages of AD (Imbimbo et al., 2010). Interestingly, in this study, we found that intranasal treatment of IFN β , a cytokine with anti-inflammatory properties, improves memory performance in the AD-modeled rats at both doses. These results add a new

insight and gain attention toward potential beneficial effects of IFN β through IN delivery in AD context. In a clinical study it has been revealed that subcutaneous injection of IFN β 1a, three times a week, in early AD patients lead to partial but not significant amelioration of cognitive deficits (Grimaldi et al., 2014). This discrepancy can be attributed to the low CNS bioavailability of IFN β following systemic administration (Dixon et al., 2016; Ross et al., 2004). As shown in previous studies IN approach provides a non-invasive and direct route of IFN β delivery into the CNS with minimal doses and less systemic side effects (Dixon et al., 2016; Ross et al., 2004; Thaney et al., 2017; Thorne et al., 2008). There is evidence showing IFN β delivery into the hippocampus following IN administration of IFN β in rats (Ross et al., 2004). Similarly, Dixon et al have indicated that intranasal administered IFN β reaches to the brain and causes reduction in the infarcted area and improvement of neurological behavior in a rat model of ischemia (Dixon et al., 2016). IFN β is a member of type I IFNs family which also includes multiple IFN α subtypes (González-Navajas et al., 2012). Both IFN β and IFN α signal through IFNAR (Ivashkiv and Donlin, 2014). Minter et al have reported that deletion of the IFNAR1, in an APP_{Sw/Ind}/PS1 Δ E9 transgenic mouse model of AD, alleviates cognitive deficits (Minter et al., 2016). These controversial results given the protective effects of IFN β in our study and protective effects of IFNAR knockout in the mentioned study may be attributed to the blockade of IFN α signaling which shares IFNAR with IFN β . In agreement with this assumption, Minter et al have shown that IFN α level but not IFN β increases in the cortex of AD mice (Minter et al., 2016). Although both IFN β and IFN α signal through IFNAR, it is demonstrated that they stimulate different biological responses in the CNS which are cell type- and context-dependent (Ivashkiv and Donlin, 2014; van Boxel-Dezaire et al., 2006). In the CNS, IFN β activity is more neuroprotective while IFN α is

preferentially neurotoxic. It has been shown that IFN α overexpressing transgenic mice develop severe neurodegeneration and cognitive decline (Akwa et al., 1998; Campbell et al., 1999). In addition, the increased level of IFN α in the CSF is responsible for cognitive deficits in the patients with Aicardi-Goutieres syndrome (Berger et al., 2007) and human immunodeficiency virus (Anderson et al., 2017; Sas et al., 2009). Similarly, the patients with cancer (Capuron et al., 2002; Scheibel et al., 2004) and hepatitis (Lieb et al., 2006; Reichenberg et al., 2005) treated with IFN α for long-term periods suffer consequently from memory disturbance and depression. In contrast, there are studies similar to our findings which have reported the protective effect of IFN β on memory performance. Ejlerskov et al demonstrated that IFN $\beta^{-/-}$ mice exhibit cognitive impairment and PD-like dementia (Ejlerskov et al., 2015). Also, it has been indicated that IFN β treatment prevents cognitive decline and slows brain atrophy in patients with RRMS (Barak and Achiron, 2002; Kappos et al., 2009; Patti et al., 2010, 2013).

It has been indicated that APP fragments trigger the production of pro-inflammatory cytokines in APP transgenic mice independently of A β (Cavanagh et al., 2013; Suh and Checler, 2002). In this study, concomitant with memory deficits, we observed a neuroinflammatory response manifested by an increase in the mRNA levels of GFAP and Iba1, as glial markers, and TNF- α , IL-1 β , and IL-6 as pro-inflammatory cytokines. In line with our findings, neuroinflammation has been found in the brains of APP_{Sw/Ind} transgenic mice (Wright et al., 2013) and AD patients (Monson et al., 2014; Parachikova et al., 2007) before A β plaque deposition, which is correlated with early memory impairments. It has been reported that neuroinflammation causes A β plaque formation (Sastre et al., 2008). Consistent with these studies, we observed A β plaques in the different regions of hippocampus. Furthermore, it has been revealed that inhibition of neuroinflammation in transgenic mouse models of AD prevents cognitive dysfunction and neuropathology (Gabbita et al., 2012; McAlpine et al., 2009; Tweedie et al., 2012). In the present study, we also showed that treatment with two different doses of IN IFN β remarkably alleviates inflammatory responses, though it was more effective at the higher examined dose. Furthermore, both doses increase the expression of IL-10. It has been shown that removal of IFNAR1 in the in vitro and in vivo models of AD does not completely eliminate neuroinflammation, but inhibits the expression of some pro-inflammatory genes (Minter et al., 2016; Taylor et al., 2014). These observed anti-inflammatory effects may be associated with the blockage of IFN α signaling through IFNAR. As mentioned above, IFN β and IFN α bind to IFNAR and subsequently activate JAK-STAT1 and JAK-STAT3 signaling pathways. Activation of the JAK-STAT1 pathway induces the expression of pro-inflammatory genes. In contrast, activation of the JAK-STAT3 pathway induces the expression of anti-inflammatory genes and also attenuates the function of JAK-STAT1 pathway (Ho and Ivashkiv, 2006; Ivashkiv and Donlin, 2014). So, the balance between STAT1 and STAT3 activity determines the relative ability of these cytokines to inhibition or stimulation of inflammation (Ho and Ivashkiv, 2006). It has been indicated that IFN α preferentially activates STAT1 in human neuroblastoma cells (Minter et al., 2014) and also can induce pro-inflammatory responses in the CNS (Hayley et al., 2013). Over-expression of IFN α in the mouse brain induces gliosis and progressive inflammatory encephalopathy (Akwa et al., 1998; Campbell et al., 1999). Studies have demonstrated that IFN α mainly elevates the expression of IL-1 β , IL-6 and TNF- α genes in the prefrontal cortex and hippocampus which could potentially mediate some of IFN α treatment-induced neuropsychiatric adverse effects such as memory impairments (Hayley et al., 2013; Hoyos-Becerra et al., 2014; Wichers et al., 2007). However, in accordance to our results, it has been reported that endogenous or exogenous IFN β attenuates gliosis, reduces the expression of pro-inflammatory mediators like IL-1 β , IL-6, and TNF- α and induces the IL-10 gene expression in the animal models of SCI (Nishimura et al., 2013; Sandrow-Feinberg et al., 2010), stroke (Kuo et al., 2016), and MS (Hou et al., 2014; Lubina-Dąbrowska et al., 2017).

5. Conclusions

Our findings showed that over-expression of mutant human APP gene in the hippocampus of adult rats induces memory impairment concomitant with glial activation, increased expression of pro-inflammatory cytokines and decreased expression of anti-inflammatory cytokine. Also based on the obtained results, IFN β IN administration attenuates memory impairment in this model of AD. The neuroprotective effect of IFN β on memory may be possibly associated with suppression of gliosis and shift from pro- to anti-inflammatory cytokines production. So, our findings suggest that IFN β may be a promising therapeutic agent to improve cognitive functions and modulate inflammatory responses in an AD-like neurodegenerative context.

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