



Cytokine-induced Neurogenesis for Alzheimer's Disease and Frontotemporal Dementia

Takuji Shirasawa^{1)*}, Luis Carlos Aguilar Cobos²⁾

1) *Ochanomizu Health and Longevity Clinic, Tokyo, Japan*

2) *Livant Neurorecovery Center, Guadalajara, Mexico*

Abstract:

Alzheimer's disease (AD) and frontotemporal dementia (FTD) are two major causes of dementia. These diseases are both progressive neurodegenerative diseases whereas no curative treatment has not yet been established. Regenerative approaches have been extensively researched for AD and FTD, but they are still in early phase of pre-clinical trials. We show here, for the first time, that cytokines such as hepatocyte growth factor (HGF), granulocyte colony stimulating factor (GCSF), insulin-like growth factors (IGFs), and progranulin (PGRN) can induce the neurogenesis of GABAergic and glutamatergic neurons in cerebral cortex and hippocampus of AD and FTD patients. We also showed the evidence, for the first time, that the atrophied hippocampi were significantly regenerated by cytokine-induced neurogenesis in AD and FTD patients, which was confirmed by MRI study and neurophysiological evaluations. We therefore explored the potentiality of cytokine cocktail treatment for AD and FTD and showed that cytokine-induced neurogenesis is a novel promising strategy to cure AD and FTD.

Keywords:

Alzheimer's disease (AD), Frontotemporal Dementia (FTD), Cytokine, Neurogenesis, Dementia

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1. HGF induces the neurogenesis of GABAergic interneurons to suppress hyper-excitability of glutamatergic neurons in Alzheimer's disease.

Alzheimer's disease (AD) is a neurodegenerative disease, but no curative treatment has not yet been established [1]. Little is known about GABAergic interneurons in the pathogenesis of AD. In animal models of Alzheimer's disease, however, aberrant GABA signaling affects fate specification of neuronal progenitors and dendrite growth of differentiating neurons in aged hippocampus [2]. Recent evidence also suggests that neural network activity is aberrantly increased in AD brains due to functional deficits of GABAergic inhibitory interneurons, resulting in hyper-excitability of glutamatergic excitatory neurons, which in turn contributes to cognitive deficits in AD [3]. Therefore, we explored here the clinical application of cytokines such as hepatocyte growth

factor (HGF), granulocyte colony stimulating factor (GCSF), insulin-like growth factors (IGFs), and progranulin (PGRN) to induce neurogenesis of GABAergic and glutamatergic neurons in AD and FTD.

HGF and its tyrosine kinase receptor, encoded by the MET cellular proto-oncogene, are expressed in the nervous system from pre-natal development to adult life, where they are involved in neuronal growth and survival [4]. Recent evidence also suggests that HGF acts on neuronal stem cells to enhance neurogenesis and exerts beneficial neuroprotective effects in neurodegenerative diseases [4]. In the development of embryonic brain, HGF is a neurotrophic factor and an axonal chemoattractant [4]. HGF-MET are also expressed in postnatal brain in hippocampus and cortex, where HGF-MET contribute to synaptogenesis and neuroplasticity of adult brain [4]. Interestingly, common "C" variant allele (rs1858830 "C") in the MET promoter region is genetically

*Corresponding author: Takuji Shirasawa, shirasawa@shirasawa-acl.net

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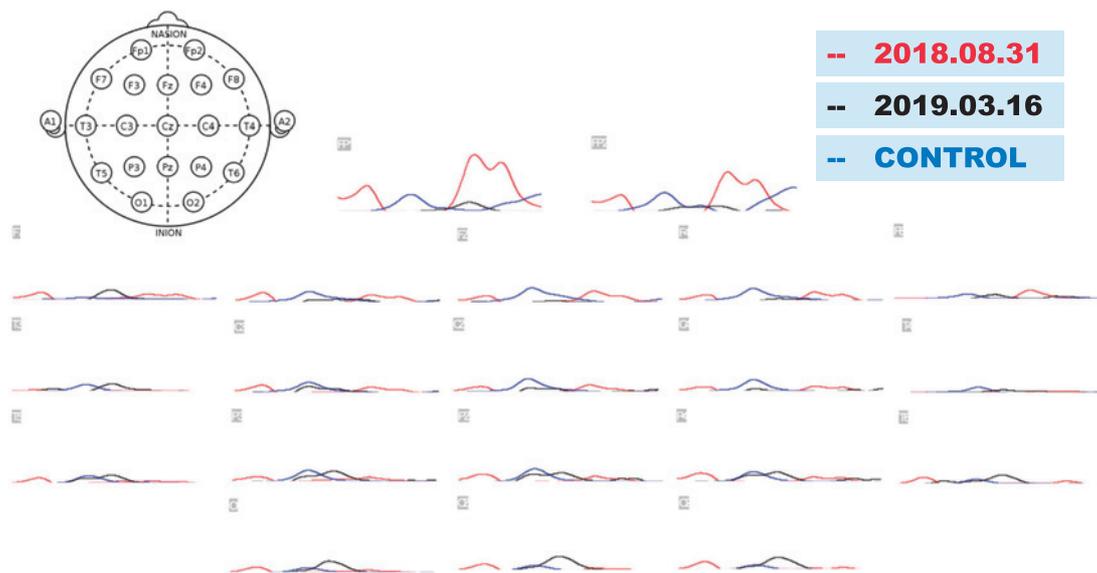


Fig. 1. Electrophysiological evaluation of p300 EEG responses before and after cytokine-induced neurogenesis of 63-year-old AD case. The position of electrodes is shown in left upper inset. Before cytokine treatment p300 EEG responses recorded on August 31, 2018, are shown (red line). After cytokine cocktail treatment p300 EEG responses recorded on March 16, 2019 (black line). Control p300 EEG responses (Blue line).

associated with autism spectrum disorder (ASD) [5], suggesting the possibility that HGF-MET play a pivotal role in neurogenesis and neural network formation of cerebral cortex associated with learning and communication. In adult hippocampus, HGF-MET also contributes to the maintenance of self-renewal and proliferation of neuronal stem cells (NSCs), suggesting the possible therapeutic application of HGF for neurodegenerative disorders such as AD [2]. It is also noteworthy that in epilepsy model animal, HGF induced the neurogenesis of GABAergic interneurons, which suppressed epileptic EEG activities and improved seizure behaviors in mice [5]. As shown Fig. 1, p300 EEG examination of 63-year-old female AD patient carrying single ApoE 4 allele (ApoE 4/3) showed the premature p300 responses in all regions of cerebral cortex (as indicated by red line) while control p300 EEG showed the single peak at around 300 msec (as shown by blue lines), suggesting that Amyloid- β ($A\beta$) accumulation in cerebral cortex may be initially toxic to GABA neurons prior to glutamatergic neuronal cell death, which then increase the excitability of glutamatergic neurons to the toxic levels in preclinical phase of AD. Interestingly, other excessive p300 responses were also observed in the delayed phase at around 600–900 msec in all regions of cerebral cortex (Fig. 1, indicated by red lines), implying that $A\beta$ deposition in cerebral cortex may not only deplete GABAergic interneurons called basket cells that inhibit dendritic input of pyramidal neurons, but also deplete GABAergic interneurons called chandelier cells that specifically inhibit axonal output of pyramidal neurons in cerebral cortex [6]. The illustrated case showed the typical abnormal p300 responses frequently observed in AD, which characteristically showed the symmetrical premature p300 responses and symmetrical delayed p300 responses in all regions of

the cerebral cortex as shown in frontopolar electrodes of Fig. 1, implying no vascular pathology involved in the neurophysiological abnormalities. We then applied HGF for the treatment of this case, the early stage of AD, to enhance the neurogenesis of GABAergic interneurons to suppress the hyper-excitability of glutamatergic neurons by using the supplement that contains HPLC-purified porcine hepatocyte growth factor. The enhanced neurogenesis was evaluated by p300 electrophysiological examination 4 months after the supplements administration. As shown in Fig. 1, the premature and delayed p300 responses disappeared in all regions of cerebral cortex after the treatment of HGF and that single p300 responses generated, albeit the peak is a bit delayed and observed at around 400–500 msec (Fig. 1 indicated by black lines), implying HGF enhanced neurogenesis of GABAergic inhibitory interneurons in cerebral cortex to inhibit glutamatergic pyramidal neurons, reverting to normal p300 physiological responses after the auditory stimuli. To this end, we have treated 69 patients diagnosed as Alzheimer's disease, from July 2018 to April 2022, by MRI and p300 neurophysiological EEG evaluation where we observed a similar improvement as illustrated in Fig. 1 for p300 EEG evaluation after the administration of cytokine cocktail containing high concentration of HGF in association with improved cognitive functions (unpublished data). This is the first report, as far as we know, that cytokines including HGF is effective for the treatment of Alzheimer's disease and promising clinical strategy to cure the progressive neurodegenerative diseases such as Alzheimer's diseases.

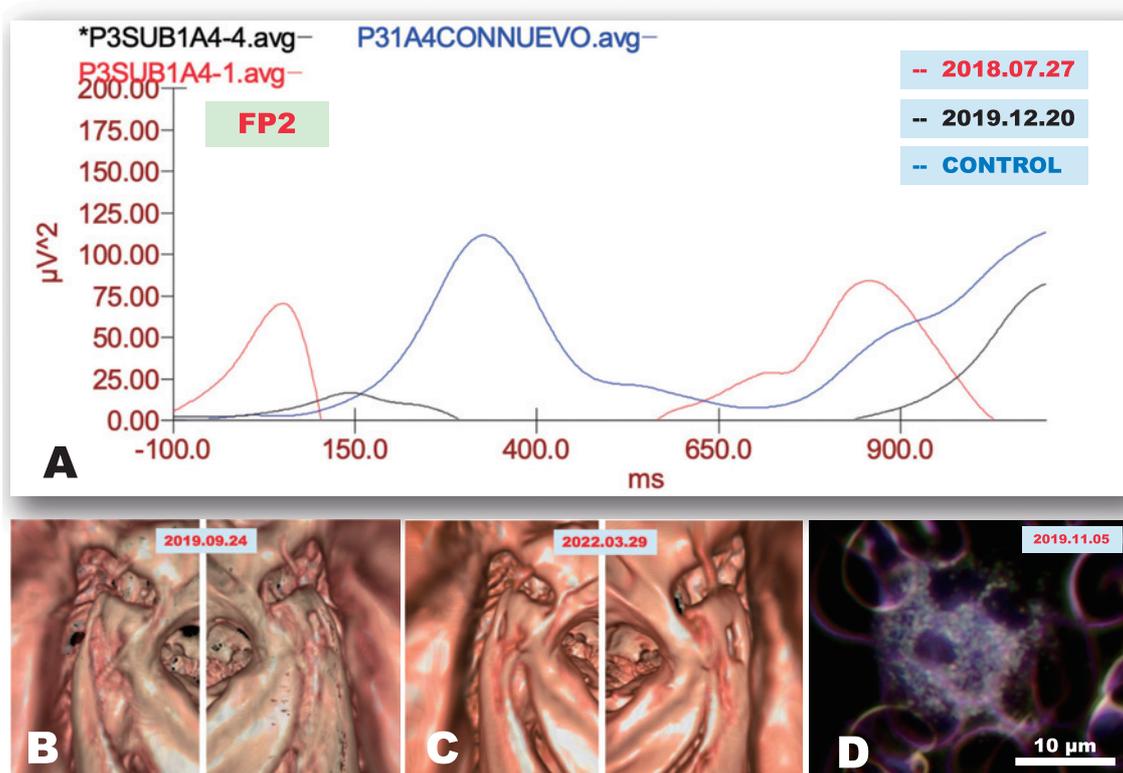


Fig. 2. A. Electrophysiological evaluation of p300 EEG responses in right frontopolar electro lead (FP2) before and after cytokine-induced neurogenesis of 73-year-old male AD case. Before cytokine treatment p300 EEG responses recorded on July 27, 2018, are shown (red line). After cytokine cocktail treatment p300 EEG responses recorded on December 20, 2019 (black line). Control P300 EEG responses (Blue line). B. 3D-structure of both hippocampi reconstructed in silico from MRI T1-weighted images with 1 mm slice recorded on September 24, 2019. C. 3D-structure of both hippocampi reconstructed in silico from MRI T1-weighted images with 1 mm slice recorded on March 29, 2022. D. Light microscopic view of a neutrophil and red blood cells from peripheral blood with dark-field microscopy. The oscillation of fine vesicles in the cytoplasm and the migration of neutrophil were recorded as movies by scanning from CCD camera equipped with Olympus Microscope.

2. GCSF activates microglia to eliminate A β in AD brain

Microglia are the resident macrophages of the central nervous system and play key roles in brain development and physiology during life and aging [7]. In AD, microglia reaction was initially thought to be incidental and triggered by A β deposits and dystrophic neurites. However, recent genome-wide association studies have established that the majority of AD risk loci are found in or near genes that are highly expressed in microglia [7], suggesting the causative role of microglia and the possibility that the activation of microglia could be the potential therapeutic strategy to cure AD by eliminating the A β deposits. Recent evidence also suggests that intracranially injected CD4 T cells activated microglia which enhanced the clearance of A β deposits with an attenuation of AD pathology in mouse models [8]. During neuronal inflammation, microglia can be activated by variety of inflammatory mediators, adjacent astrocytes, and neurotransmitters. In neuronal inflammation of the brain, astrocytes secrete cytokines such as GCSF and CCL11 to activate microglia [9]. GCSF is an endogenous neuronal hema-

topoietic factor that displays robust in vitro and in vivo neuroprotective activities [10]. Recent evidence also showed that GCSF improves memory and neurobehavior in A β induced experimental model of AD [10].

73-year-old male patient carrying ApoE4 in both alleles (ApoE 4/4) was diagnosed as AD by MRI and p300 EEG examination in July 2018 (Fig. 2A, 2B). In the first evaluation of p300 EEG, premature and delayed p300 responses were observed before cytokine treatment (Fig. 1A, red line). After the administration of cytokine cocktail containing HGF, GCSF, and IGFs, the premature and delayed p300 responses disappeared and new p300 response appeared with peak at 150 msec (Fig. 2A, black line), suggesting the neurogenesis of GABAergic interneurons and the suppression of the hyper-excitability of glutamatergic neurons in right frontopolar region (FP2). As also illustrated in Fig. 2B, brain MRI showed the atrophy in anterior parts of both hippocampi recorded in September 2019, which were significantly regenerated in March 2022 (Fig. 2C). Since ApoE4 accelerates the accumulation of A β concomitant with AD progression, the hippocampus regeneration shown in this case suggests the clearance of A β deposits in AD brains. We

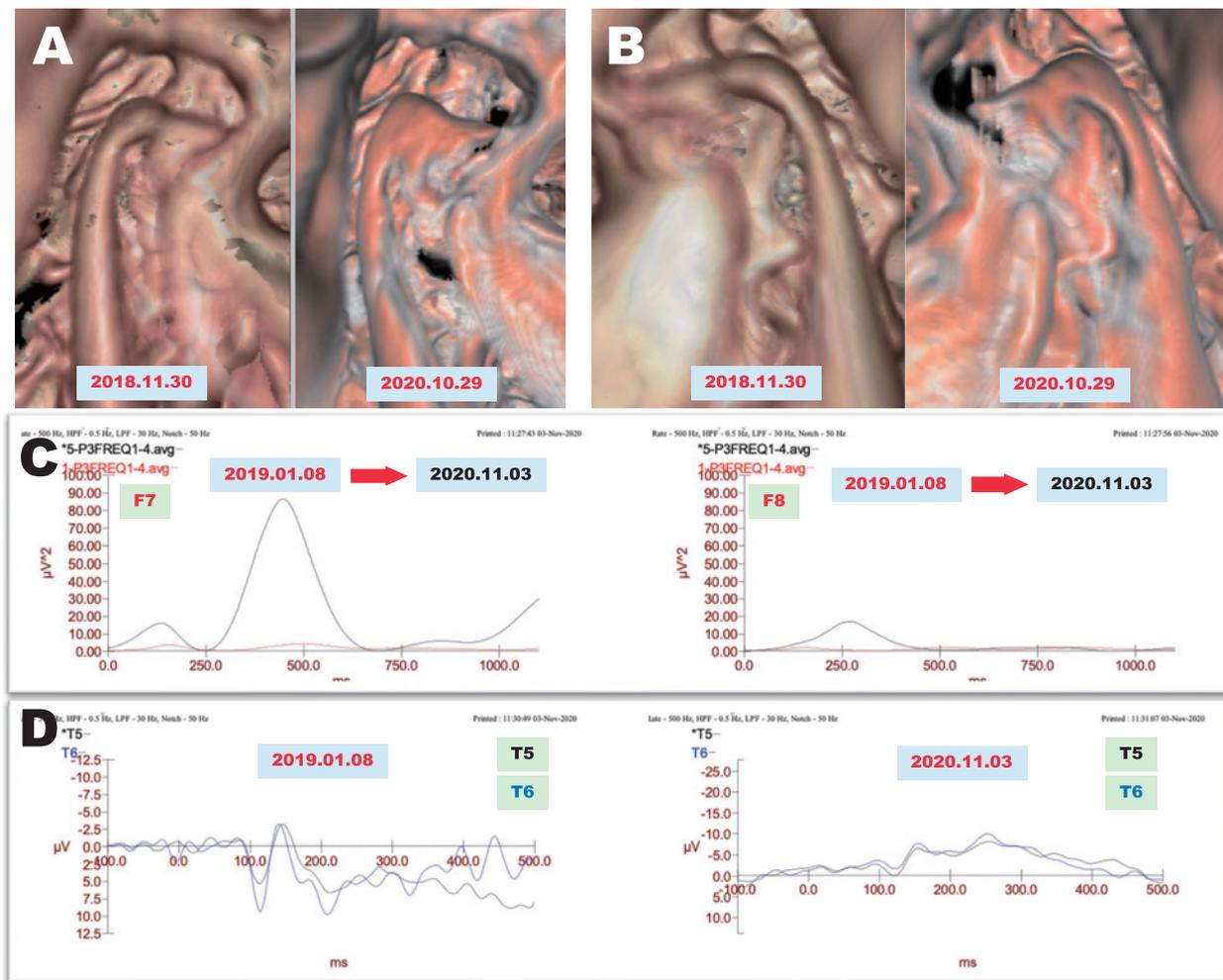


Fig. 3. A. 3D-structure of left hippocampi reconstructed in silico from MRI T1-weighted images with 1 mm slice recorded on November 30, 2018, before cytokine cocktail treatment and left hippocampi recorded on October 29, 2020, after 2 years cytokine cocktail treatment. B. 3D-structure of right hippocampi recorded on November 30, 2018, before cytokine cocktail treatment and left hippocampi recorded on October 29, 2020, after 2 years cytokine cocktail treatment. C. Electrophysiological evaluation of p300 EEG responses in both frontolateral electro lead (F7, F8) before and after cytokine cocktail treatment. Before cytokine treatment, p300 EEG responses recorded on January 08, 2019, are shown by red line. After cytokine cocktail treatment p300 EEG responses recorded on November 03, 2020, are shown by black lines. D. Flash visual evoked potentials compared between left temporal posterior (T5) and right temporal posterior (T6) before cytokine cocktail treatment recorded on January 08, 2019 (Fig. 3D, left panel) and after cytokine cocktail treatment recorded November 03, 2020 (Fig. 3D, right panel).

then studied if the neutrophils collected from peripheral blood was activated by cytokines under live microscopy where we found that the cytoplasm of neutrophils is filled with oscillating vesicles and migrating on slides for phagocytosis under live microscopic view (Fig. 2D). As microglia and neutrophil share the common receptor for GCSF, activation of neutrophils in peripheral blood might implicate the activation of microglia in the brain, suggesting the possibility that administered GCSF activated brain microglia to eliminate A β deposits with the concomitant reduction of A β neurotoxicity.

3. PGRN induces hippocampal neurogenesis in FTD

PGRN is a multi-functional protein. Full-length form of PGRN shows trophic and anti-inflammatory activity while the proteolytic cleavage of PGRN generate granulin peptides that promote inflammatory activities [11]. The mutation of PGRN gene is closely associated with the development of a subtype of FTD, specifically tau-negative, ubiquitin-immunoreactive neuronal inclusions-positive, and TDP-43-positive neuropathology of FTD [11]. Also, mutations that reduce PGRN levels increase the risk for developing AD, Parkinson’s disease (PD), and limbic predominant age-related TDP-43 encephalopathy, as well as exacerbate the progression of amyotrophic lateral sclerosis (ALS) [11].

PGRN is a competitive inhibitor of TNF- α which binds to TNFRs so that PGRN could also be applied to treat inflammatory diseases such as arthritis and osteoarthritis [12,13]. PGRN also suppresses neutrophil chemotaxis caused by TNF- α released from brain endothelial cells after cerebral ischemia-reperfusion and inhibits the expression of pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6 through TNFR2 [14–16]. Despite of its anti-inflammatory effects, PGRN also promotes neurites outgrowth and cell survival in the CNS [17,18]. The down-regulation of PGRN decreases synapse density in primary hippocampal culture while PGRN restores dendritic spine density and neurogenesis in hippocampus [19]. In this context, elevating PGRN levels is an attractive therapeutic strategy for wide variety of neurodegenerative diseases that cause dementia such as FTD, AD, and PD.

We show here, for the first time, that cytokine cocktail containing HPLC-purified PGRN prepared from oyster, was effective for the treatment of 66-year-old FTD patient (Fig. 3). The patient was diagnosed as FTD by MRI and p300 EEG examination on January 08, 2019 (Fig. 3A, 3C, 3D). In the first evaluation of p300 EEG, p300 responses were almost flat with peak voltage less than 5 μV^2 in both left lateral frontal lobe (F7) and right lateral frontal lobe (F8) (Fig. 3A, 3B, red lines). After administration of cytokine cocktail containing PGRN for almost 2 years, new p300 EEG responses were observed in both lateral frontal lobes (Fig. 3C). However, the newly generated p300 EEG responses in F7 and F8 showed asymmetrical pattern with respect to the voltage and timing, which is frequently observed in FTD (Fig. 3C). Flash visual evoked potentials recorded in January 2019 showed marked asymmetry between right posterior temporal lobe (T5) and left posterior temporal lobe (T6) (Fig. 3D, left panel). After administration of cytokine cocktail containing PGRN, asymmetry between T5 and T6 significantly improved in November 2020 (Fig. 3D). As also illustrated in Fig. 3A, 3B, brain MRI showed the atrophy in anterior parts of both hippocampi recorded in November 2018, which were significantly regenerated in October 2020 (Fig. 3A, 3B). It is interesting to note that the bigger p300 EEG response with higher voltage was recorded in the left side of frontal lobe while the better regeneration was observed in the anterior part of left hippocampus (Fig. 3A) in comparison with right hippocampus (Fig. 3B). This is the first report, to our knowledge, that cytokine cocktail including PGRN was applied to regenerate the atrophied hippocampi and frontal lobes of FTD.

Still undefined is the biological mechanisms of PGRN on the survival and regeneration of neurons and control of microglial activation in neurodegenerative diseases as well as the aging brains [11]. Recent evidence suggests that decrease of PGRN levels due to genetic mutations impairs lysosomal dysfunction so that neuronal ability to degrade pathologically accumulated protein such as TDP-43, impaired in FTD brains [11]. In our clinical experience on more than 100 cases of dementia patients who were treated with cy-

tokine cocktail including PGRN, symptom improvement is more rapid and robust than cytokine cocktail including HGF, suggesting that action sites of PGRN is not only the neurogenesis of new neurons, but also the repair of neuronal dysfunctions with abnormal protein accumulations such as TDP-43.

We are still in early phase of clinical application for cytokine cocktail treatment for neurodegenerative diseases. The most important question is whether we can stop the neurodegenerative processes of AD and FTD with cytokines or suppress the progression of degenerative pathology. The second important question is whether we can reverse the cognitive functions by enhancing the neurogenesis with cytokines even if we fail to stop the pathological progression of AD or FTD. Further efforts and studies will be definitively needed for answering these two important and fundamental questions.

Disclosure of potential conflicts of interest

None

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None.

Declarations of interest

None.

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