Short Communication

Anti-Inflammatory Gene Therapy Improves Spatial Memory Performance in a Mouse Model of Alzheimer’s Disease

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Accepted 12 November 2021
Pre-press 9 December 2021

Abstract. The immune system plays a critical role in neurodegenerative processes involved in Alzheimer’s disease (AD). In this study, a gene-based immunotherapeutic method examined the effects of anti-inflammatory cellular immune response elements (CIREs) in the amyloid-\(\beta\) protein precursor (A\(\beta\)PP) mouse model. Bi-monthly intramuscular administration, beginning at either 4 or 6 months, and examined at 7.5 through 16 months, with plasmids encoding Interleukin (IL)-10, IL-4, TGF-\(\beta\) polynucleotides, or a combination thereof, into A\(\beta\)PP mice improved spatial memory performance. This work demonstrates an efficient gene therapy strategy to downregulate neuroinflammation, and possibly prevent or delay cognitive decline in AD.

Keywords: Alzheimer’s disease, amyloid-\(\beta\) protein precursor, genetic therapy, immunotherapy, interleukin-10, interleukin-4, neuroinflammation, spatial memory, transgenic mice, transforming growth factor beta

INTRODUCTION

Alzheimer’s disease (AD), the most common form of dementia, is not a normal part of aging, but rather a chronic neurodegenerative pathology associated with neuroinflammation, extracellular amyloid-\(\beta\) (A\(\beta\)) plaques, and hyperphosphorylated tau, which leads to progressive cognitive decline in older adults [1–3]. The specific cause of AD remains unclear, but it may collectively involve the accumulation of activated microglia, astrocytes, and proliferative T cells which target extracellular filamentous abnormal A\(\beta\) protein deposits in the brain [4–7]. Although most neurodegenerative diseases are not classically considered autoimmune, in some instances, chronic neuroinflammation in aging can exacerbate a progressively declining innate immune system leading to further neuronal damage [8, 9].

The adaptive immune system can be broadly classified into two types of inflammatory activity: cellular and humoral (antibody). Among cytokine responses, proinflammatory T helper type 1 (Th1) (i.e., interferon-gamma (IFN-\(\gamma\)), tumor necrosis factor (TNF-\(\alpha\)), IL-1, IL-2, IL-12), an anti-inflammatory Th2 (i.e., IL-4, IL-5, IL-10, IL-13), Th3 (TGF-\(\beta\)), and Th17 are involved in neurodegenerative disease and could be targeted for therapy. Moreover, anti-inflammatory CIREs such as IL-10 and TGF-\(\beta\) play a critical role in neurodegenerative autoimmune diseases such as multiple sclerosis, Parkinson’s disease, and amyotrophic lateral sclerosis [10–14]. Although immunotherapeutic clinical trials were previously halted, emerging work continues to corroborate the importance of T cell recognition and autoimmune susceptibility in the etiology of AD [15–17].
For patients experiencing mild cognitive impairment, an elevated presence of proinflammatory TNF-α concurrent with decreased anti-inflammatory TGF-β levels was observed, resulting in a greater risk conversion towards AD [18]. Similarly, TNF-α and other proinflammatory cytokines such as IL-1β and IL-6 were reported to impact anti-inflammatory processes and increase amyloid brain deposition in transgenic AD mice [19–21], with the latter cytokine driving blood-brain barrier dysfunction [22]. Anti-inflammatory cytokines, IL-4 and IL-10, have also garnered therapeutic interest due to their immunomodulatory role in the autoreactive T cell repertoire of neurodegenerative diseases [23–25]. Researchers evaluating IL-4 and IL-10 polymorphisms in patient populations have reported that a relative paucity of the genetic disruptions in these TH2 cytokines increases susceptibility to developing AD [26–31]. Therefore, the specific role these and other anti-inflammatory cytokines play in preventing or ameliorating neurodegeneration in AD not only warrants further investigation, but also requires new experimental approaches.

For example, gene-based technologies could provide a promising therapeutic strategy to ameliorate neurodegenerative disease due to administration ease, an efficacious and safe profile, and long-lasting effects [32–35]. Previous Yoo laboratory gene transfer work, examining anti-inflammatory response using a clinically relevant allergen to induce experimental autoimmune hearing loss, was successful in controlling autoimmune reaction severity through suppression of Th1-type proinflammatory responses and inducing IL-10-secreting regulatory T cells [36]. This non-toxic naked DNA delivery technique suggests exogenous IL-10 could restore immunological homeostasis by suppressing the autoimmune response and generate an endogenous regulatory IL-10 profile. Since chronic inflammation appears to trigger T cell-mediated autoimmune disease, the present study assessed whether anti-inflammatory CIRE gene therapy could also improve spatial memory performance in the amyloid-β protein precursor (AβPP) mouse, and thus prevent or delay AD onset.

MATERIALS AND METHODS

Transgenic animals

Transgenic mice (Tg-2576) containing the K670N/M671L (AβPP) Swedish double mutation, which leads to familial early onset AD [37], were obtained from The Jackson Laboratory (Bar Harbor, ME, U.S.A.) and maintained at the University of Tennessee, Memphis animal facility after experimental approval by the Institutional Animal Care and Use Committee of the University of Tennessee.

Plasmid DNA preparation and transfection reagents

Polynucleotide constructs under a simian virus 40 promoter encoding a CIRE were used: IL-4, IL-10, TGF-β (i.e., GenBank Accession No. M13982, 55 SEQ ID NO:12), (M57627, SEQ ID NO:14), (M60316, SEQ ID NO:16), respectively. Expression of CIRE naked DNA plasmids utilized the pVAX1 vector (Invitrogen, Carlsbad, CA) and cytomegalovirus promoter/enhancer sequences. A control vector without the CIRE genes was developed by digesting related plasmid DNA with EcoRI, followed by ligating the agarose gel-purified vector fragment. Large-scale purification of all plasmid DNA was conducted with Endo Free Plasmid Maxi kits (Qiagen, Carlsbad, CA). Methodology was reported [38–42], with dosage effective at least 1–5 weeks after injection, and adopted from previous studies [36, 43]. Male and female AβPP mice were bi-monthly, intramuscularly injected with either 100 µg of a blank vector in 100 µl of phosphate-buffered saline for control or the same amount of naked DNA encoding CIREs. Mice were used or maintained until age 60 weeks, then sacrificed, brains removed, snap-frozen in liquid nitrogen, and stored at −80°C.

Spatial memory performance evaluation in untreated and treated AβPP mice

Spatial learning and memory were assessed using the Morris Water Maze task [44] in a circular tank (80 cm wide, 80 cm deep) with a non-distinct submerged central platform (15 cm wide, 1 cm below opaque water, 23°C) (Fig. 1). All trials were recorded with an overhead camera. Maximum swim time for each trial was 90 s followed by a 20-s platform rest. Each mouse was trained for five days, four trials per day with randomized starting points. Probe trials were performed without the platform 30 min after the last trial. Mice were released opposite the target quadrant and allowed 60 s to swim. Following retraining (day 7), the platform was moved to the opposite quadrant for reversal training (days 8–10). A retention test was conducted 30 min after the last acquisition trial, and latency (seconds to platform) was registered.
Fig. 1. Evaluation of spatial memory in AβPP mice using the Morris Water Maze. The MWM is an intermittently sensitive test to evaluate reference memory performance for AβPP mice [47].

| Testing Age (month) | 7.5 | 8  | 9  | 9.5 | 11 | 13  | 16  |
|---------------------|-----|----|----|-----|----|-----|-----|
| Normal Untreated Mouse | 6   | 6  | 6  | d.n.r | d.n.r | 6   | 6   |
| AβPP control vector | 120 | 120 | 160, 52 | 144, 52 | 144 | 220, 100 | 62  |
| AβPP + TGF-β | 23  | 23 | 80, 70 | 72, 66  | 78, 6 | 80, 30 | 14  |
| AβPP + IL-10 | 15  | 15 | 8, 7, 15 | 8, 7   | 2, 7  | 1, 1, 8 | 3, 4 |
| AβPP + IL-4 | d.n.r | d.n.r | 10   | 10   | 10  | 4   |
| AβPP + (IL-10 + IL-4) | d.n.r | d.n.r | d.n.r | d.n.r | 1, 2 | 3   |
| AβPP + (IL-10 + TGF-β) | d.n.r | d.n.r | d.n.r | d.n.r | 45, 44 | d.n.r |

Normal Untreated Mice (n = 5), AβPP control vector (n = 10), TGF-β (n = 11), IL-10 (n = 14), IL-4 (n = 5), IL-10 + IL-4 (n = 3), and IL-10 + TGF-β (n = 2) latencies for individual mice were registered and indicated by numerical values. d.n.r., data not recorded.

(47) Combined cytokine-treated animals were only tested at 13 and 16 months of age.

Data analysis

Latency times with animals (n ≥ 7) administered with CIREs beginning at 6 months were measured at specific ages (7.5, 9.5, 11, 13, 16 months) and analyzed by Welch’s t-test and one-way ANOVA with Tukey HSD Post Hoc in R open source software (https://cran.case.edu/) [45]. Threshold values of p = 0.05 were considered statistically significant.

RESULTS

Early and late TGF-β, IL-10, or IL-4 gene therapy prevent and ameliorate AβPP mice memory deficits

Mice injected bi-monthly with naked DNA encoding TGF-β, IL-10, or IL-4 beginning at 4-months of age (8 & 9 months columns), when hippocampal lesions begin to appear in the AβPP model, and tested in the MWM at 8 or 9 months (when lesions fully form [46]) reduced latency times to platform compared to age-matched AβPP mice receiving a control blank vector (Table 1). Interestingly, AβPP mice receiving either TGF-β, IL-10, or IL-4, or a CIRE gene combination bi-monthly beginning at 6 months, and examined at 7.5 through 16 months, also reduced latency-to-platform behavior. Overall, AβPP mice administered TGF-β, IL-10, or IL-4 naked DNA performed significantly better compared to AβPP controls (p = 0.014, 0.002, and 0.002, respectively; Fig. 2). No significant difference was observed between Normal Untreated (not illustrated) and IL-10 or IL-4 treated mice (p = 0.777, 0.194, respectively).

DISCUSSION

The present study demonstrates that gene-based immunotherapy, with pleiotropic anti-inflammatory cytokines IL-10, IL-4, or TGF-β improves spatial memory performance in a mouse model of AD. AβPP mice treated bi-monthly, beginning at age
risk profile. Dose based on the patient’s metabolic and stratified utilization the appropriate biomolecule, adjuvant and an example, non-viral immunotherapy for AD couldPrecise therapeutic intervention may be required. As a chronically inflamed system may unintentionally advances, introducing exogenous Th2 cytokine into viral vector technology for neuronal system delivery aberrant innate immune amyloidosis [57–59]. While accumulation and impaired memory, resulting in modification increased hippocampal and cortical Aβ in two different AD models, IL-10- and IL-4-AAV induced an anti-inflammatory response from an alternative activated macrophage phenotype while stimulating microglia and astrogliosis [54, 55]. A separate group also reported that AAV-IL-4 induced an anti-inflammatory response from an alternative activated macrophage phenotype while stimulating microglia and astrogliosis [54, 55]. However, in two different AD models, IL-10- and IL-4-AAV modification increased hippocampal and cortical Aβ accumulation and impaired memory, resulting in aberrant innate immune amyloidosis [57–59]. While viral vector technology for neuronal system delivery advances, introducing exogenous Th2 cytokine into a chronically inflamed system may unintentionally exacerbate and accelerate neurodegeneration [60–63]. Precise therapeutic intervention may be required. As an example, non-viral immunotherapy for AD could utilize the appropriate biomolecule, adjuvant and dose based on the patient’s metabolic and stratified risk profile [64, 65].

Although anti-inflammatory gene therapy, in the present report, improved overall spatial memory deficits in AβPP mice, it remains unclear whether the corresponding cytokine levels increased in the periphery and/or neuronal tissue with the given dose. Previous experiments in the Yoo laboratory confirmed, through harvested splenocyte cultures and cochlear histology of IL-10−/− mice with experimental autoimmune hearing loss, that intramuscular injection of 100 μg IL-10 DNA provided sufficient peripheral and cranial IL-10 production [36]. Interestingly, proinflammatory-induced neurodegeneration in an AD rat model was also alleviated in a dose-dependent manner with TGF-β [66]. Notably, combinatorial TGF-β/IL-10 plasmid DNA immunotherapy has already been explored to treat humoral autoimmune diseases [67]. Safe, novel, and optimized gene-based neuroimmunotherapies will be essential as drug development advances [68–70].

In oncology, ‘cytokine synergy’ infers that combined therapeutic potency is greater than any of the individual cytokines alone [71]. Intriguingly, older mice administered IL-10 + IL-4, and to a lesser extent IL-10 + TGF-β, could augment spatial memory in AβPP mice (Table 1), suggesting synergistic anti-inflammatory AD amelioration. Additionally, plasmid delivery of DNA encoding IL-10/IL-4 prevents autoimmune diabetes in nonobese diabetic mice [72], while fusion protein treatments with these Th2 cytokines alleviates inflammatory pain [73, 74]. Currently, inflammation in these concomitant diseases exacerbates cognitive decline [75–79], but synergistic cytokine gene therapy could provide health benefits not only for the aging population, but society as a whole.

Finally, AD mouse models exhibit anxiety, age/gender performance variability, elevated retinal Aβ, increased proinflammatory Th1 cytokines, and early-onset biomarker absence, which may confound behavioral data [53, 80–85]. Despite their putatively dubious nature, gene-based immunotherapy research in transgenic mice should continue exploring enhanced delivery systems and promotors, complementing adjuvants, and confirmation of experimental results in other AD models, such as rabbits, where artificially-induced risks factors are closer to human AD [86–90]. Furthermore, clinical trials for neurodegenerative disease involving naked DNA require more investment due to plasmid DNA biocompatibility, lower manufacturing cost, efficient production, and storage stability [91–93]. In summary, the present study provides an efficient

![Fig. 2. AβPP mice treated with TGF-β, IL-10, or IL-4 improves spatial memory. AβPP mice injected bi-monthly, beginning at 6 months, with either TGF-β (n=8) or IL-10 (n=10) and tested at 7.5, 9.5, 11, 13, and 16 months showed significant difference compared to AβPP mice receiving control vector (n=7) \( F(2,22)=22.69, p<0.001 \). AβPP mice administered IL-4 (n=4) also reduced swim latency compared to TGF-β (p=0.011). Mean±S.E.M.; Welch’s t test; *p<0.05, #p<0.01.](image-url)
strategy of preventing/delaying AD onset through down regulation of chronic inflammation using gene therapy.

ACKNOWLEDGMENTS

Funding was provided by Hearing Health Foundation (formerly, Deafness Research Foundation) New York, NY, USA; Veterans Administration Medical Center Memphis, TN, USA; and R01 Grant from NIH-NIDCD-RO1-DC005010-01-A2.

Related United States Patent: 8,247,385 B2 AUG, 21, 2012.

Plasmid DNA encoding murine IL-10 was a gift from Barry T Rouse, PhD, University of Tennessee, Knoxville, TN; experimental support provided by Mohammad Habiby Kermany, MD/PhD

Gregory J. Villareal, Ph.D. provided editorial support.

The author’s disclosure is available online (https://www.j-alz.com/manuscript-disclosures/21-5270r2).

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